THE UNIVERSITY OF ALBERTA

ALTERED BEHAVIOUR LEADING TO SELECTIVE PREDATION OF AMPHIPODS INFECTED WITH ACANTHOCEPHALANS, WITH SPECIAL REFERENCE TO *POLYMORPHUS PARADOXUS*

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "Altered behaviour leading to selective predation of amphipods infected with acanthocephalans, with special reference to *Polymorphus paradoxus*" submitted by William Mack Bethel, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

ABSTRACT

The freshwater amphipods, *Gammarus lacustris* and *Hyalella azteca*, infected with acanthocephalan and cestode larvae were used to test Holmes and Bethel's (in press) hypothesis that some parasites have adopted the evolutionary strategy of altering the behaviour of their intermediate hosts so as to increase the vulnerability of the latter to predation by the definitive host.

Uninfected *G. lacustris* and *H. azteca* are strongly photophobic, negatively phototactic, and found in the bottom ooze and heavily vegetated areas of the lakes. They evade disturbance by diving to the bottom, often burrowing into the sediment or crawling under debris; the primary directive for this response is their negative phototaxis. The behaviour and distribution of gammarids infected with cystacanths of *Polymorphus contortus* (Acanthocephala: Polymorphidae) and the cysticercoids of *Lateriporus mathevossianae*, *L. skrjabini*, and *L. clerci* (Cestoda: Dilepidae) were indistinguishable from those of the uninfected amphipods.

Gammarids infected with *P. marilis* were photophilic but negatively phototactic. Hyalellids infected with *Corynosoma constrictum* were strongly photophilic, showing a distinct preference for areas with the highest level of illumination. A significant proportion of the infected hyalellids were positively phototactic before and after disturbance, but the number of positive individuals was significantly decreased by disturbance.

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INTRODUCTION

> In gaining an entrance into their hosts the parasitic worms seem to show the most astounding knowledge of the activities and habits of life of the host. Had they ability to see, hear, and reason it seems doubtful whether they could exhibit a more diabolical cunning to gain their ends than they do now.
>
> - George R. LaRue (in Szidat, 1969)

> The study of behaviour has not yet developed as a recognized area in helminthology, yet accumulating evidence suggests more and more that in every stage, highly specialized, complex behaviour patterns occur in response to exacting requirements of each species. The careful and critical analysis of adaptive behaviour for each life cycle stage, and the elucidation of trigger mechanisms including chemical, hormonal, sensory, and neurosensory stimuli, undoubtedly will provide challenging areas of inquiry for the intellectually curious helminthologist.
>
> - Martin J. Ulmer (1971)

The quotations cited above emphasize the highly evolved, complex mechanisms utilized by parasites for gaining entry into their intermediate and definitive hosts. One critical stage of their life cycles, often the most difficult, is the transmission to the definitive host. A large proportion of the parasitic helminths are transferred through an intermediate host, which is ingested by the definitive host. It is not surprising, therefore, that parasites use the behavioural patterns of their intermediate and definitive hosts, particularly in respect to their predator-prey interactions, to enhance that transmission.

One aspect of predator behaviour, as it applies to prey selection, Tinbergen's (1960) "specific searching image," may be important in the success of some parasites. Mueller (1971) recently found that search

images were the most important factor determining the selection of differently colored mice by hawks. His tests showed that oddity was nearly as important, and conspicuousness was much less important. Croze (1970) showed that the searching image plays a similarly important role in prey selection by carrion crows. The importance of search images in one of the systems investigated in the present study will be discussed later.

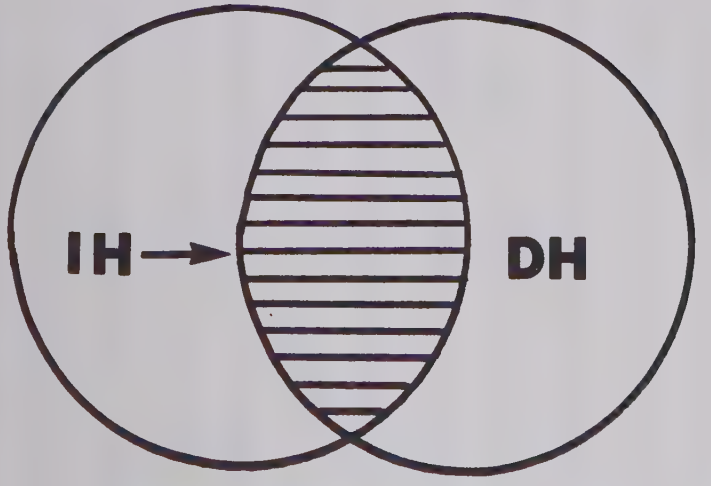
Holmes and Bethel (in press) have reviewed some of the more important characteristics of predator-prey relationships, and deduced evolutionary strategies which parasites may adopt to increase the probability of their transmission to the definitive host. The evolutionary strategies which were presented include reduced stamina, increased conspicuousness, disorientation, and altered responses to environmental stimuli of the intermediate hosts. It was pointed out that the degree of overlap between the habitat (or feeding niche) of the predator (the definitive host) and that of the prey (the intermediate host) would determine the success of the strategy and, therefore, the kind of strategy which would be evolved. Where the habitat of the intermediate host is enclosed within the feeding niche of the definitive host (Fig. 1), the first three strategies (reducing the stamina, increasing the conspicuousness, and disorientation) would be sufficient. However, when the habitats are only partly overlapping, the strategy would be more complicated, and should also involve altering the behaviour of the infected intermediate host so that the latter would move into the area of overlap (as indicated by the arrow in Fig. 1). An extensive review of the evidence that parasites have adopted these strategies was presented.

Fig. 1. Strategic options of a parasite when the habitat of the intermediate host is within the habitat (feeding niche) of the definitive host, and when the habitats are only partly overlapping.

ENCLOSED HABITATS

Decrease Stamina
Reduce Response
to Predator
Greater
Conspicuousness

OVERLAPPING HABITATS



Alter Response to Increase Overlap

The most intriguing behavioural alterations, and the ones most conducive to laboratory investigation, are those involving altered responses to environmental stimuli. One classic example of this strategy involves formicine ants and metacercariae of the sheep liver fluke, *Dicrocoelium dendriticum* (reviewed in Carney, 1969; Holmes and Bethel, in press). Hohorst and his co-workers (Hohorst and Graefe 1961, Hohorst and Lämmler 1962, Hohorst 1964) showed that most of the cercariae ingested by the ant penetrated into the abdominal haemocoele and encysted, but that one (or occasionally two or three) migrated to the subesophageal ganglion, where it encysted close to the nerves to the mouthparts. These metacercariae were not infective to definitive hosts, but were associated with a marked change in the behaviour of the infected ants. Infected ants were attached to the tops of blades of grass or leaves of plants during the cooler parts of the day, when uninfected ants had returned to the nest. Anokhin (1966) and Grus (1966) showed that the behavioural change was temperature-dependent, and possibly also light- or humidity-dependent, with the ants migrating up the vegetation and grasping the plants with their mandibles as the temperature decreased in early evening, remaining torpid in such locations throughout the night, and becoming reactivated with rising temperatures during the morning. At midday, their activity is apparently similar to that of uninfected ants. This behavioural pattern keeps the infected ants near the top of the vegetation during the early morning and late evening grazing periods of their ungulate definitive hosts, but allows them to move to less exposed areas during the hot, dry midday. This is an elegant example of the modification of the behaviour of the host to increase the probability of being

ingested accidentally by the definitive host.

Carney (1969) discovered several behavioural peculiarities in carpenter ants, *Camponotus* spp., carrying metacercariae of another dicrocoelid trematode, *Brachylecithum mosquensis*. Infected ants contained at least one metacercaria in or around the supraesophageal ganglion, plus several others in the tissues of the markedly enlarged gaster. A much higher proportion (91%) of infected worker ants were found in a collection made in an open, rocky area than in a collection from an adjacent wooded area (9%). Infected ants could typically be found slowly circling, or remaining motionless for hours on the surfaces of rocks. Infected ants did not respond to sudden changes in light intensity produced by shading them. Carpenter ant workers in temperate regions are normally strongly photophobic (Wheeler 1910). Tapping the rock on which an infected ant was circling or resting produced a "brief stirring. The ant would return to its previous pattern of behaviour within a short time, however" (Carney 1969). The response of infected ants to temperature may also be altered, since Carney found only infected ants active outside of the nests in late fall. These behavioural modifications would obviously increase the vulnerability of the infected ants to predation by insectivorous birds, including robins, the definitive hosts for *B. mosquensis*.

The *D. dendriticum*/ant/sheep system (and possibly the *B. mosquensis*/ ant/robin system) is an excellent example of how altered responses to environmental stimuli can produce an overlap between the habitat of the infected intermediate host and the feeding niche of the definitive host. Three other possible examples, involving marine bivalves and trematode and

cestode larvae are given in Holmes and Bethel (in press). It is clear from their review of these examples, and examples of the existence of other strategies, that the number of speculations greatly exceeds the number of investigations. In no case has either the specific response of the infected intermediate host or the actual vulnerability to predation been subjected to critical experimental evaluation.

In order to test the hypothesis that parasites have adopted evolutionary strategies for specific predator-prey systems, one must ideally have a single species of intermediate host which, in a single community, harbours different species of helminths which are specific to different definitive hosts having different predatory behaviour (feeding niches). Such a situation exists in two Alberta lakes which have been the major study areas for the parasitology group at the University of Alberta for several years, Cooking and Hastings Lakes. Both lakes have large populations of *Gammarus lacustris* Sars (Amphipoda: Gammaridae) and *Hyalella azteca* (Saussure) (Amphipoda: Talitridae), which serve as intermediate hosts for five species of polymorphid acanthocephalans and several species of hymenolepid and dilepid cestodes (Denny 1969, Podesta and Holmes 1970a, b). Some of these helminths are specific to one or two of the several species of waterfowl which occur on the lakes (Table 1). In addition, Denny (1967) and others working at the lakes have noticed that gammarids infected with cystacanths of one of the acanthocephalans, *Polymorphus paradoxus* Connell and Corner, 1957, but not uninfected gammarids or those infected with other helminths, were often attached to or closely associated with floating material. These observations suggested the existence of an altered pattern of behaviour in at least one of the host-parasite systems.

Table 1. Local intermediate and definitive hosts of the parasites included in the study

Parasite	Intermediate hosts	Definitive hosts	
		Main	Auxiliary
Acanthocephala			
Polymorphus paradoxus	*Gammarus lacustris*[1]	Muskrats, Beavers,[2] Mallards[3]	Shovelers[3]
P. marilis	*G. lacustris*[1]	Lesser Scaup[1,4]	Ruddy Ducks,[4] Redheads,[1] Red-necked Grebes[3]
P. contortus	*G. lacustris*[1] and *Hyalella azteca*[5]	Various Dabbling and Diving Ducks[1,3,5]	
Corynosoma constrictum	*H. azteca*[5]	Various Dabbling and Diving Ducks[3,4,5]	
Cestoda			
Lateriporus mathevossianae	*G. lacustris*[1]	White-winged Scoters[1]	
L. skrjabini	*G. lacustris*[1]	Lesser Scaup[1,4]	Redheads, Canvasbacks[1,4]
L. clerci	*G. lacustris*[1]	Ring-billed,[1] Franklin's[1] and Bonaparte's Gulls[6]	Red-necked Grebes[1]

Authors: 1 = Denny (1969); 2 = Connell and Corner (1957); 3 = Unpublished records; 4 = Graham (1966); 5 = Podesta and Holmes (1970); 6 = Hair and Holmes (1970).

Thus the characteristics of the larval helminth/amphipod/waterfowl systems at Cooking and Hastings Lakes appeared to be excellent for testing the hypothesis outlined above. My overall objectives with respect to the principal hypothesis were: (1) to search for behavioural alterations in as many of the larval helminth/amphipod combinations as possible; (2) to clearly define the responses involved in the behavioural alterations; (3) to show that the behavioural alterations are different and specific to each larval helminth/amphipod combination; (4) to show that the specific behavioural alterations render the intermediate hosts (amphipods) more vulnerable to the specific definitive hosts (waterfowl) of the parasites and to their particular feeding habits or predatory behaviour.

The principal subject of the investigation was *G. lacustris* infected with the cystacanths of *P. paradoxus* (Fig. 2). Other combinations which were used were *G. lacustris* infected with the cystacanths of *P. marilis* Van Cleave, 1939, and *P. contortus* (Bremser, 1821) Travassos, 1926, or the cysticercoids of *Lateriporus mathevossianae* Ryzhikov and Gubanov, 1962, *L. skrjabini* Matevosian, 1946, and *L. clerci* (Johnston, 1912) (Cestoda: Dilepidae), and *H. azteca* infected with the cystacanths of *Corynosoma constrictum* Van Cleave, 1918 (Polymorphidae). These particular combinations were chosen because of their availability and because the helminth larvae were all identifiable, by their size, shape, and color, through the carapaces of living amphipods. The cysticercoids of *L. skrjabini* and *L. clerci* cannot be distinguished *in vivo*; they were therefore treated as *Lateriporus* spp.

The local intermediate and definitive hosts for the helminths

Fig. 2. A cystacanth of *Polymorphus paradoxus* in the haemocoele of *Gammarus lacustris*.

included in the study are presented in Table 1. With the exception of *P. paradoxus*, the life cycles of all had been established experimentally, prior to the present study, by Denny (1969) or Podesta and Holmes (1970a). Connell and Corner (1957) described *P. paradoxus* adults from muskrats (*Ondatra zibethica*) and beavers (*Castor canadensis*) and speculated that *G. lacustris* were carrying the larval stages. Denny (1969) described the cystacanths from *G. lacustris*, identifying them on the basis of their similarity with the adult forms in hook size and formula, but did not attempt to complete the life cycle in the laboratory. I have completed the life cycle experimentally using eggs from gravid or mature females from naturally infected muskrats and mallard ducks (*Anas platyrhynchos*) to infect laboratory-reared *G. lacustris*, and have obtained gravid females from experimental infections in muskrats, hamsters, chickens, and laboratory reared mallards.

The infection in gammarids can be detected, and, by their size and shape, can be distinguished from the other polymorphids at an early-acanthella stage (19-21 days at 19-20 C). Their development can be followed, *in vivo*, through to the cystacanth stage (40-42 days at 19-20 C). Therefore, the acanthellae of *P. paradoxus* could also be used in the behavioural study.

GENERAL METHODS

THE STUDY LAKES

Field observations and collections were made at Cooking and Hastings Lakes, Alberta, 17 and 20 miles (27 and 32 km) southeast of Edmonton, respectively. Both are highly productive, eutrophic lakes. Their general limnology was described by Kerekes (1965).

In Cooking Lake, the water is extremely turbid due to a high concentration of suspended clay particles and moderately large populations of phytoplankton. The bottom is soft and covered by a layer of ooze throughout most of the lake. Accordingly, the rooted aquatic vegetation, which consists mainly of *Potamogeton* sp., is sparse and limited to the edge of the shoreline and very shallow areas. Although the invertebrate fauna has not been studied specifically, the most abundant macroscopic species include *G. lacustris*, *H. azteca*, corixids, chironomid and other insect larvae, leeches, oligochaetes, cladocerans, copepods, and ostracods. The amphipods are distributed throughout the bottom ooze of the lake, but are concentrated along the shoreline and in shallow bays. The vertebrate fauna of the lake is almost totally comprised of migratory aquatic birds, which utilize the lake for breeding and/or staging area. Among the more important are mallards, shovelers (*Anas clypeata*), blue-winged teal (*A. discors*), lesser scaup (*Aythya affinis*), white-winged scoters (*Melanitta deglandi*), eared grebes (*Podiceps caspicus*), and Franklin's and Bonaparte's gulls (*Larus pipixcan* and *L. philadelphia*). Very few muskrats or beavers have been sighted in the lake.

In comparison, Hastings Lake is deeper, less turbid, and has a

more firm bottom sediment. The light penetration is greater, but is usually reduced by extensive algal populations during the summer. The lake supports a wider, more dense zone of rooted vegetation along the shoreline. In addition to *Potamogeton* spp., *Myriophyllum* spp. and *Ceratophyllum* sp. are well established in the lake. The shorelines of both lakes are dominated by cattails (*Typha latifolia*) and reeds (*Phragmites communis*). The invertebrate fauna is basically the same as that in Cooking Lake. The distribution of *G. lacustris* and *H. azteca*, however, appears to be restricted to the heavily vegetated areas. The presence of fish in the open part of the lake may account for the distribution of the amphipods. Kerekes (1965) found northern pike (*Esox lucius*) and yellow perch (*Perca flavescens*) in the lake; their numbers, however, have declined greatly since his study and are presently very low. The aquatic bird populations are similar to those of Cooking Lake, except that red necked grebes (*P. grisegena*) are much more abundant. Also, there are significant numbers of muskrats and beavers in Hastings Lake.

COLLECTION, IDENTIFICATION, AND MAINTENANCE OF MATERIAL

Amphipods

Infected and uninfected amphipods were collected from random dip-net samples taken at the two study lakes. Gammarids infected with *P. paradoxus* were also collected from floating material (as a result of one of their behavioural alterations--clinging) in the lakes. The amphipods were returned to the laboratory, and gammarids infected with the cystacanths of *P. paradoxus* (hereafter referred to as *P.p.*), the acanthellae (the mid-acanthella stage of Butterworth, 1969) of *P. paradoxus* (*P.p.*A), the cystacanths of *P. marilis* (*P.m.*), the cystacanths of

P. contortus (*P.c.*), the cysticercoids of *L. mathevossianae* (*L.m.*), the cysticercoids of *L. skrjabini* and *L. clerci* (*L.* spp.) and uninfected gammarids were placed in separate aquaria. Amphipods containing heavy infections (more than two *P.p.*, or more than three or four of the other helminths) were not used to avoid complicating effects (e.g., reduced viability, sluggishness) not pertinent to the study. Natural infections of the helminths rarely exceeded one per amphipod (mean intensity of *P.p.* in 1970-1971 was 1.1; overall mean for the other helminths was 1.2).

The darker cuticle of *H. azteca* made differentiation of the larvae in the haemocoele more difficult. Only the infections with *C. constrictum* (*C.c.*) were distinguishable (and abundant) enough to be used; even so, cystacanths could not be differentiated from acanthellae. Infected hyalellids were therefore held in the laboratory for a period well in excess of the interval of development between the acanthella and cystacanth stages (11 days--Podesta and Holmes, 1970a) before they were used in tests.

The amphipods were held in an environmental control room in 10, 15, or 80 gallon aquaria at 19-20 C with a photoperiod of 14 hours of light and 10 hours of darkness. Each aquarium was provided with mud bottom and some floatage, such as wooden sticks and aquatic vegetation from the lakes, to serve as cover. The water of the aquaria was aerated constantly by one or two air stones; the aeration was kept at a rate which would not disturb the swimming or other movements of the amphipods. The turbidity of the water was kept as consistent with the study lakes as possible. The water was never filtered and frequently was replenished with lake water which had been sieved through a moderately fine mesh screen. The sides of the aquaria were covered with black cardboard.

These measures kept the levels of illumination in the aquaria comparable to those in the study lakes. They were also important in maintaining a rather diverse fauna in the aquaria, which served as a natural food source for the amphipods. The natural food was supplemented with brewer's yeast and lettuce.

Vertebrates

The mallards and scaup which were used in the study were raised in the laboratory from eggs collected from nests on the islands of Hastings Lake. The ducks were kept in large pens provided with small water troughs. They were fed *ad libitum* on a mash diet, and were never starved before a test. The ducks were all 4 to 10 months old when used.

Laboratory-reared muskrats were not available. The three which were used in the study were live trapped, two from Hastings Lake, and one from Wabamun Lake, Alberta. They were kept in large L-shaped cages with the tail of the L immersed in a water-filled tub. A wooden nest box was provided in each cage for shelter. The cages were maintained at 18-19 C in a temperature controlled room, and fed *ad libitum* on a combination of oatmeal, lettuce, carrots, and apples.

EXPERIMENTAL INFECTION TECHNIQUES

Gravid *P. paradoxus* females were obtained from naturally infected mallards or muskrats, or from laboratory infected hamsters. Embryonated eggs were mixed with brewer's yeast in 20-cm finger bowls. Thirty or 40 uninfected gammarids were exposed to the mixture in the dark for 30-40 minutes. This procedure usually produced a 10% yield of single-larva infections, which were the only ones used. Longer periods of exposure to the eggs and yeast usually produced heavy multiple infections which could

not be used.

These laboratory infected gammarids were used only in the experiments dealing with the development of altered behaviour and the infectivity of cystacanths.

LABORATORY APPARATUS

Behavioural Studies

Three basic types of apparatus were used. In the first (the light-dark choice aquarium), the top and sides of one-half of a 5½-gallon glass aquarium were covered with black cardboard, and a 60-watt lamp in a 15-cm reflector was positioned 25 cm above and directly over the center of the aquarium (Fig. 3), producing light and dark zones with a minimal twilight zone. The aquarium was provided with a mud bottom 1-2 cm deep and an equal amount of floatage (a 10 x 1.5 cm wooden stick and 10-cm strand of *Potamogeton* sp.) in each zone. Tests were run in a darkened environmental control room.

The same aquarium was modified for bottom light tests by covering the top with black cardboard and restricting the layer of mud to the bottom of the dark zone (Fig. 7). The lamp was positioned 25 cm below the center of the aquarium.

The second apparatus (an alternating light system) was a 14-gallon, frameless, plexiglass aquarium with a 100-watt bulb in a 15-cm reflector placed 25 cm above and below the center of the aquarium. The light sources were connected to a three-way switch, so that the direction of the light could be changed rapidly or shut off completely. The aquarium was filled with clear, dechlorinated water. Tests were run in a darkroom.

A horizontal gradient of illumination was produced in a glass

Fig. 3. The light-dark zone aquarium.

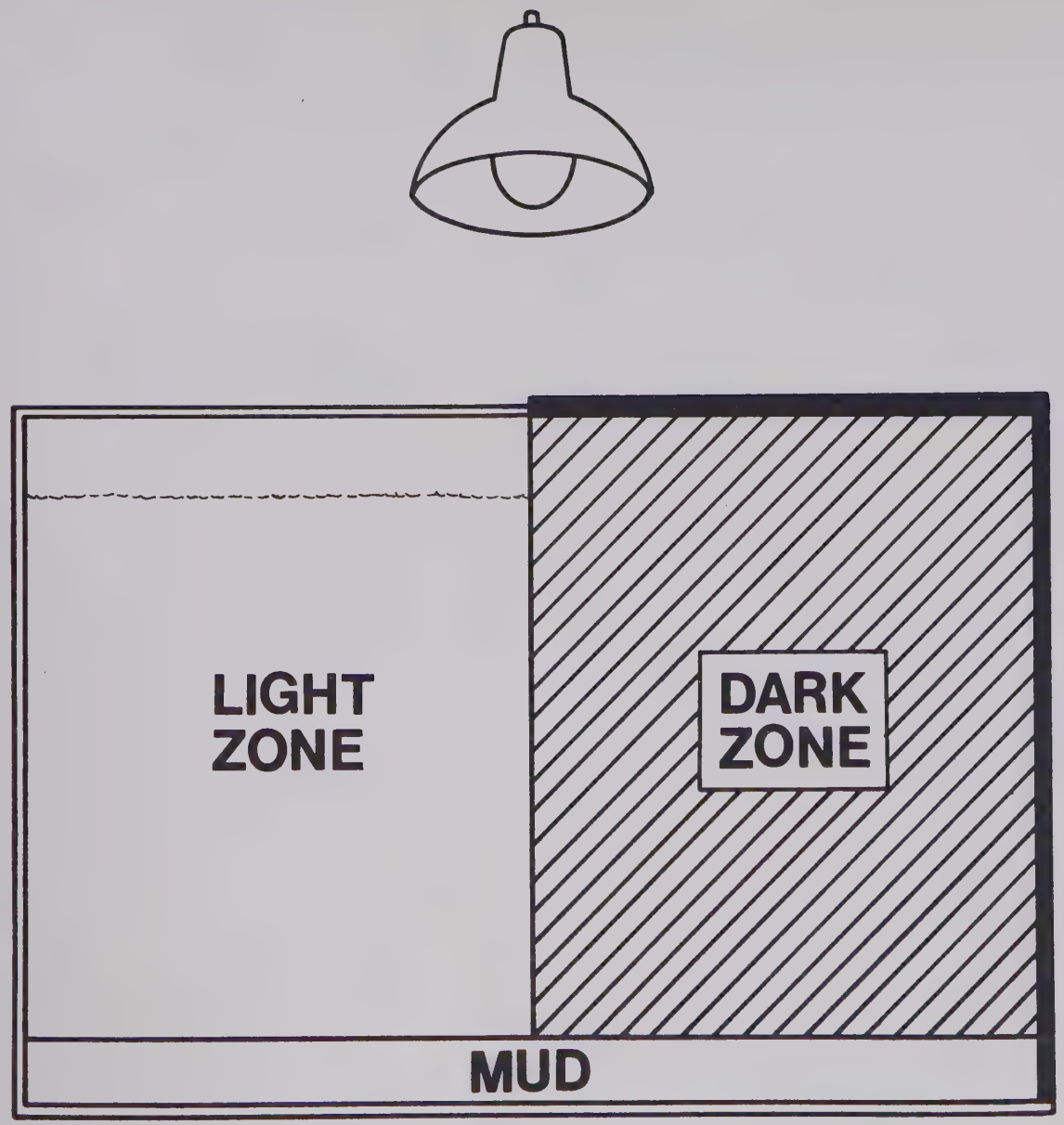
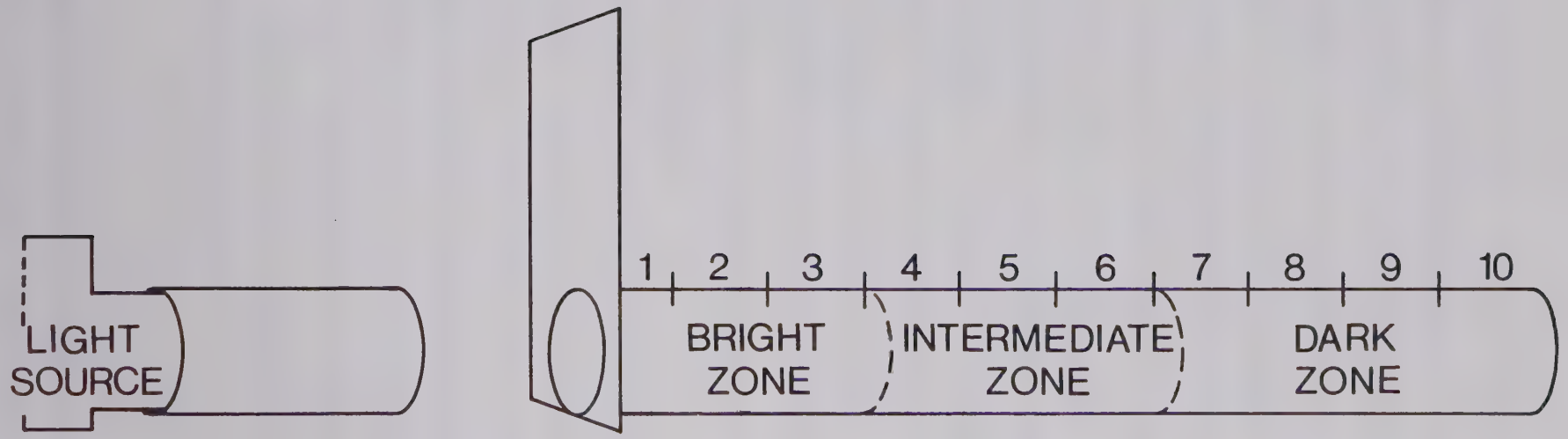

LIGHT
ZONE
DARK
ZONE
MUD

Fig. 4. The horizontal illumination gradient apparatus.


LIGHT
SOURCE
1
2
3
4
5
6
7
8
9
10
BRIGHT
ZONE
INTERMEDIATE
ZONE
DARK
ZONE

cylinder 5 ft (1.5 m) long and 5 in (12.7 cm) in diameter, closed except for an opening approximately 1.25 cm in diameter in the upper surface of one end, and completely filled with aerated turbid water, similar to that in Cooking Lake. The light beam from a Kodak slide projector with a 500-watt lamp was focused on one end of the cylinder through a 35-cm cardboard tube. The cylinder was shielded from extraneous light from the projector by a 43 x 61 cm piece of black cardboard (Fig. 4). The cylinder was marked off in ten 6-in (15 cm) sections, and the distance between the projector and the end of the cylinder (approximately 25 cm) was adjusted to produce a distinct gradient of illumination such that a gammarid was just visible in section 6. Sections 1-3 then represented a "bright zone," 4-6 an "intermediate zone," and 7-10 a "dark zone" (Fig. 4). The light was visually adjusted on each testing day so that the level of illumination in each zone remained reasonably consistent. The tests were performed in a darkroom.

During all tests and observations, except the horizontal illumination gradient tests, the water of the aquarium was aerated by an air-stone placed at the center of the side of the aquarium opposite the observer. The aeration was kept at a rate which did not disturb the amphipods. The water was maintained at the same temperature as the holding aquaria (19-20 C). In preliminary experiments, the size and position of the lamps used in the light experiments were shown to have no measurable effect on the water temperature.

Predation Studies

The predator tests were performed in two tanks, a shallow one approximately 1.8 x .6 x .3 m deep, and a deeper one 1.2 x .7 x .6 m deep.

The tanks were lined with a fine mesh net to facilitate recovery of the surviving amphipods at the end of each test. A mud bottom and approximately the same amount of floatage--one 15 x 4 cm wooden stick (or two equal to the same area), two 10-cm pieces of reeds, and two 15-cm strands of *Potamogeton* sp., *Ceratophyllum* sp., or *Myriophyllum* sp. (not available for the first two tests, but used in every test thereafter)--were provided for each test. The tanks were filled to within a few centimeters of the top with dechlorinated water. The temperature, turbidity, and pH of the water were kept as consistent with the conditions in the study lakes as possible. In order to determine the effects of the testing procedure and conditions on the survival of the amphipods, two groups of 50 *P.p.*-infected and 50 uninfected gammarids were left alone in the tanks for a 24-hour period. All of the gammarids were recovered alive at the end of each test.

The .6 m deep tank was modified for the use of the muskrats. A wooden box, 28.7 x 48 x 38 cm deep, with a two-level interior, accessible by entrances at the bottom, front, back, and one side, was mounted at one end of the tank, about 3 cm above water level. The box and open area of the tank were enclosed by chicken wire. The muskrats were left in the tank for at least 24 hours before the tests began and observed frequently during this period. An ample supply of their normal laboratory food items (see above) was available in a pan at the side of the box before and during the tests. The usual floatage (see above) was also provided, and the vegetation replenished at the beginning of the test period if necessary. At the end of the tests, the interior of the box, as well as the net, was checked for gammarids.

During each test, the animals were observed as closely as possible without disturbing their performance. Two observation positions were available when the tests were held outside: one was about 1.6 m directly above one end of the tanks, and the other about 5 m from the side of the tanks. The indoor tests were performed in an observation room fitted with one-way glass. Three of the tests involving mallards were filmed.

FILMS

A 16-mm research film was made to illustrate some of the behavioural alterations of *P.p.*-infected gammarids, normal behaviour of uninfected gammarids, and the relationship of the behavioural alterations with mallards in predator tests. Many of these behavioural patterns and reactions are otherwise very difficult to illustrate. The film is referred to periodically throughout this thesis; it is available through the Department of Zoology, University of Alberta, or through the author.

RESPONSES OF INFECTED AND UNINFECTED AMPHIPODS TO LIGHT AND DISTURBANCE

Two parallel approaches were used to study the behaviour of infected and uninfected amphipods. In the study lakes, careful observations were made on their behaviour and distribution. In the laboratory the behaviour patterns suggested by the field observations were substantiated under controlled conditions and then experimentally dissected into specific responses.

Previous workers have reported both *G. lacustris* and *H. azteca* to be negatively phototactic (Holmes 1901, Phipps 1915), and distributed primarily in the benthic and heavily vegetated zones of lakes, where light intensities are low (Mennon 1969, Oakland 1969, Hargrave 1970). In view of the probable influence of light on the normal distribution of these amphipods, and Denny's (1967) observations on the presence of gammarids infected with *P.p.* on floating material in the upper, lighted zone at Cooking Lake, the initial experimental studies were centered on responses of the amphipods to light, and designed so that the influence of light could be evaluated and compared with the influences of other environmental factors, such as gravity, oxygen content, or other factors associated with the water surface.

FIELD OBSERVATIONS

Whenever possible, the behaviour of infected and uninfected amphipods encountered at the study lakes was observed and recorded. Special efforts were made to study associations of amphipods with floating

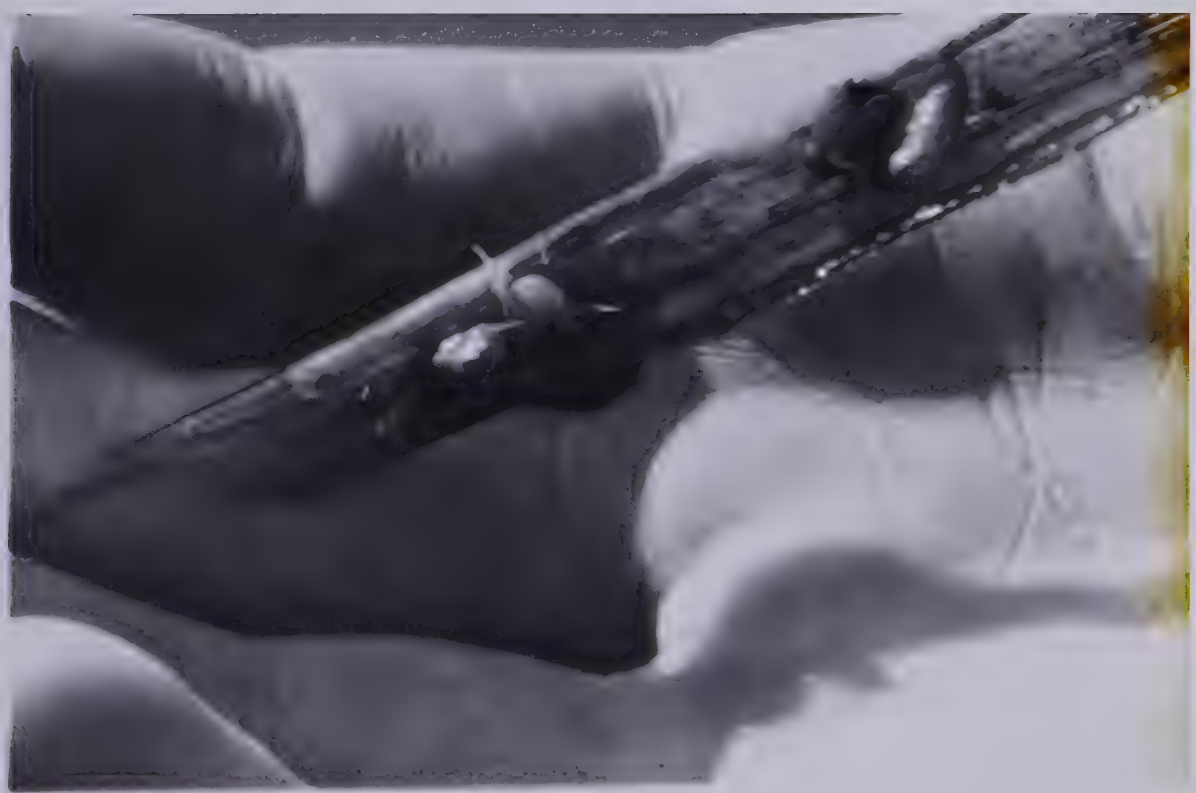
vegetation and other material at the water surface.

Uninfected amphipods were often found at the surface among the leaves of emergent vegetation, and occasionally at the sides and undersurfaces of floating plant material or dead waterfowl. When the material or the surrounding water was disturbed, the amphipods immediately dove. Identical responses were exhibited by 24 gammarids infected with *P.m.* and 5 infected with *L.* spp. These were the only gammarids infected with helminths other than *P.p.* seen at the surface.

Numerous *P.p.*-infected gammarids were found on surface material, but their behaviour and mode of association with the floating material were quite different. These gammarids were never observed to dive when disturbed, but clung persistently onto the material with their gnathopods, and remained clinging even after they were shaken or lifted out of the water (Fig. 5). Those individuals which appeared to be loosely associated with floating material before it was inspected immediately grasped at it and clung to it when disturbed.

Clinging gammarids are firmly locked onto the substrate with both pairs of gnathopods. Their posterior ends are curled ventrally and forward, and their lateral or ventral surfaces are tightly pressed against the substrate. This position is characteristic of clinging gammarids and easily distinguishable from that of a resting or feeding gammarid. The tenacity of the clinging is such that the gammarids cannot be shaken loose, but must be forcibly removed, often resulting in the loss of a gnathopod. When clinging gammarids were left undisturbed in the laboratory, they remained clinging for 10-30 minutes. The clinging and the curled position gradually relaxed until the gammarids appeared to

Fig. 5. *Gammarus lacustris* infected with *Polymorphus paradoxus* clinging onto a reed shell.



be more loosely attached, maintaining their position with the first pair of gnathopods. They eventually released completely and swam away. Any disturbance during the period of relaxation, however, re-intensified and prolonged the clinging.

When *P.p.*-infected gammarids were touched or otherwise disturbed while swimming, they usually turned and attempted to cling onto the object which touched them. If they were unable to cling, they swam to the top of the water and began "skimming" along the surface.

The skimming gammarids rapidly dig or grasp at the air-water interface with their gnathopods, in an action so pronounced that it gives the impression of an effort to get out of the water. They sometimes appear to be caught by surface tension, but when pushed under the surface they swim back up and resume the skimming. The obvious surface disturbance created by this behaviour pattern is shown particularly well in the film. The skimming occurs whenever *P.p.*-infected gammarids are disturbed and unable to cling onto material, or when they are dislodged from clinging, and continues until they encounter something to which they can cling.

Uninfected gammarids and ones infected with helminths other than *P.p.* show a different, but possibly related, clinging behaviour when exposed to certain unnatural conditions. When a group of gammarids are taken out of the water with a dip net, they initially move about very rapidly in effort to crawl through or out of the net. After being held out of the water for a few minutes their activity decreases and some cling onto the netting. When the net is placed back into the water, the clinging gammarids release and dive. Holding the gammarids out of the water for longer periods, and/or agitating them, seems to increase the

tenacity of the behaviour, so that they remain clinging for some time after being submerged. This "clinging" is obviously different from that of *P.p.*-infected gammarids in that the body of the gammarid is not tightly curled and pressed against the substrate. In addition, uninfected gammarids were never observed to cling on vegetation, reeds, wooden boards, or my hand, substrates to which *P.p.*-infected gammarids readily cling.

Although no quantitative data were kept on the numbers of uninfected gammarids found at the surface, their numbers were very low compared with their numbers in random samples. The numbers of gammarids infected with helminths other than *P.p.* were also very low at the surface. However, the numbers of *P.p.*-infected gammarids at the surface were remarkably high in comparison with the very low extensities of *P.p.* in gammarids taken in random dip net samples. For example, the extensities of infection in Cooking Lake in August and September, 1969, were 4.1% and .9% (Tokeson, personal communication), yet up to 35 *P.p.*-infected gammarids could be found clinging to each of several dead waterfowl which had been placed in the same sampling site and left floating for only 10-20 minutes (Table 2). Even greater differences were noted at Hastings Lake. Hundreds of infected gammarids were found clinging along the waterline of a wooden boat dock and on floating debris when random population samples taken at the same time (but in a different bay) showed *P.p.* extensities of 0% (July) and .4% (September). These and other examples are presented in Table 2.

Disturbing the bottom or the vegetation appeared to increase the numbers of *P.p.*-infected gammarids at the surface. *P.p.*-infected gammarids were often seen skimming in the trails left by people walking

Table 2. *Gammarus lacustris* infected with *Polymorphus paradoxus* found clinging to surface material in comparison with those in dip net samples from the same lake

Lake	Date	Dip net sample	Clinging		
			No.	Time	Surface material
Cooking Lake, Alberta	Aug. 1969	4.1% of 303	70	15 min[+]	Dead waterfowl (3)
	Aug. 1969	4.1% of 303	106	20 min[+]	Dead waterfowl (5)
	Aug. 1969	4.1% of 303	33	45 min[o]	Reed shells and emergent vegetation
	Sept. 1969	0.9% of 302	145	20 min[+]	Dead waterfowl (7)
	Sept. 1969	0.9% of 302	45	10 min[+]	Dead waterfowl (4)
	July 1970	0 of 579	38	15 min[o]	Emergent vegetation
	Aug. 1970	Not sampled	60	15 min	Dead waterfowl (3) and off wooden dock
	Aug. 1970	Not sampled	35	10 min[+]	Dead waterfowl
Hastings Lake, Alberta	Sept. 1970	0.4% of 226	257	1¼ hr[o]	Floating debris and wooden dock
	Sept. 1970	0.4% of 226	130	1 hr[o]	Wooden dock

[+]Approximate time waterfowl were left floating before they were inspected for clinging gammarids.

[o]Approximate time spent searching for clinging gammarids.

through the vegetation or along the shores of the lakes, and after working in such areas for short periods, one could often find several gammarids clinging onto the hairs of his leg.

RESPONSES IN A LIGHT-DARK CHOICE AQUARIUM

In preliminary laboratory observations, in which the positions of infected and uninfected amphipods were checked after they had been left in an aquarium providing a choice between light and dark zones (Fig. 3) for periods of 30 minutes to 10 hours, *P.p.*-infected gammarids and *C.c.*-infected hyalellids were always found among the floatage in the upper 3 cm of the lighted zone; uninfected amphipods were never found in the lighted zone.

In order to study their distribution and activity in the aquarium more thoroughly, the amphipods were observed for 1-hour periods and the time spent by each amphipod in the upper 3 cm of the light zone (ULZ), which constituted 1/9 of the light zone, and in the remainder of the light zone (RLZ) was recorded on a multiple channel event recorder. Amphipods not observed in the light zone were assumed to be in the dark zone (DZ). The amphipods to be tested were introduced into the light-dark zone aquarium (described earlier) in total darkness; the light was then switched on and they were allowed to acclimate for at least 1 hour before the observations began. During the initial observations, only five amphipods could be effectively observed simultaneously; later, as it became possible to predict the activity of some amphipods, eight or nine could be used. One *P.p.*-infected gammarid, one or two *C.c.*-infected hyallelids, and equal numbers of uninfected control amphipods were used in the basic test, which was replicated ten times, using new amphipods in

each test. Gammarids infected with *P.p.*A, *P.m.*, and *L.m.* were run with the basic test as often as possible. Each of these combinations was run at least five times, using different individuals.

The time, in seconds, spent by each amphipod in the ULZ and the total time spent in the lighted zone (TLZ) were transformed to arcsine values and tested for homogeneity by the analysis of variance (Steel and Torrie 1960). Where there was a significant between-group variance, group means were compared by Duncan's New Multiple Range test at the 5% level of probability (Steel and Torrie 1960). The differences between infected and uninfected hyalellids were so obvious that they were not analyzed statistically; the ULZ values of infected hyalellids, however, were compared statistically with those of the gammarid groups.

The results for each amphipod were compared (by a paired T-test) with the times expected to be spent in the various zones if the activity were distributed randomly throughout the aquarium. The expected values for the ULZ were calculated on the basis of the total time spent by each amphipod in the lighted zone; i.e., 1/9 x TLZ-time of amphipod x = expected ULZ-time of amphipod x.

Results

The mean recorded time, in seconds, spent in each zone by infected and uninfected amphipods is presented in Table 3. Analysis of the observations revealed highly significant relationships between the type of infection and time spent in the three areas (Table 4). The data are shown graphically in Figure 6. It is obvious that each group prefers a specific zone or zones within the choice aquarium, and that none was distributed randomly.

Table 3. Proportion of time spent by infected and uninfected amphipods in different areas of a light-dark choice aquarium

		Time (mean + S.E.), in seconds, spent in:			
	N*	ULZ**	RLZ**	TLZ**	DZ**
Gammarus lacustris					
Uninfected	10	16± 12	40± 24	56± 35	3543± 35
Polymorphus paradoxus cystacanths	10	2615±272	534±218	3150±227	450±227
P. paradoxus acanthellae	6	42± 27	94± 83	136±104	3262±132
P. marilis	6	282± 77	3318± 77	3600± 0	0
Lateriporus mathevossianae	5	114± 62	224± 72	338±131	3464±104
Hyalella azteca					
Uninfected	12	0	0	0	3600± 0
Corynosoma constrictum	12	3570± 25	30± 25	3600± 0	0
Random***		202	1598	1800	1800

*Number of 1-hour observations.

**ULZ = Upper 3 cm of Light Zone; RLZ = Remainder of Light Zone; TLZ = Total Light Zone; DZ = Dark Zone.

***Random values calculated from relative volume of each zone.

Table 4. Analysis of proportional time spent by infected and uninfected amphipods in a light-dark choice aquarium

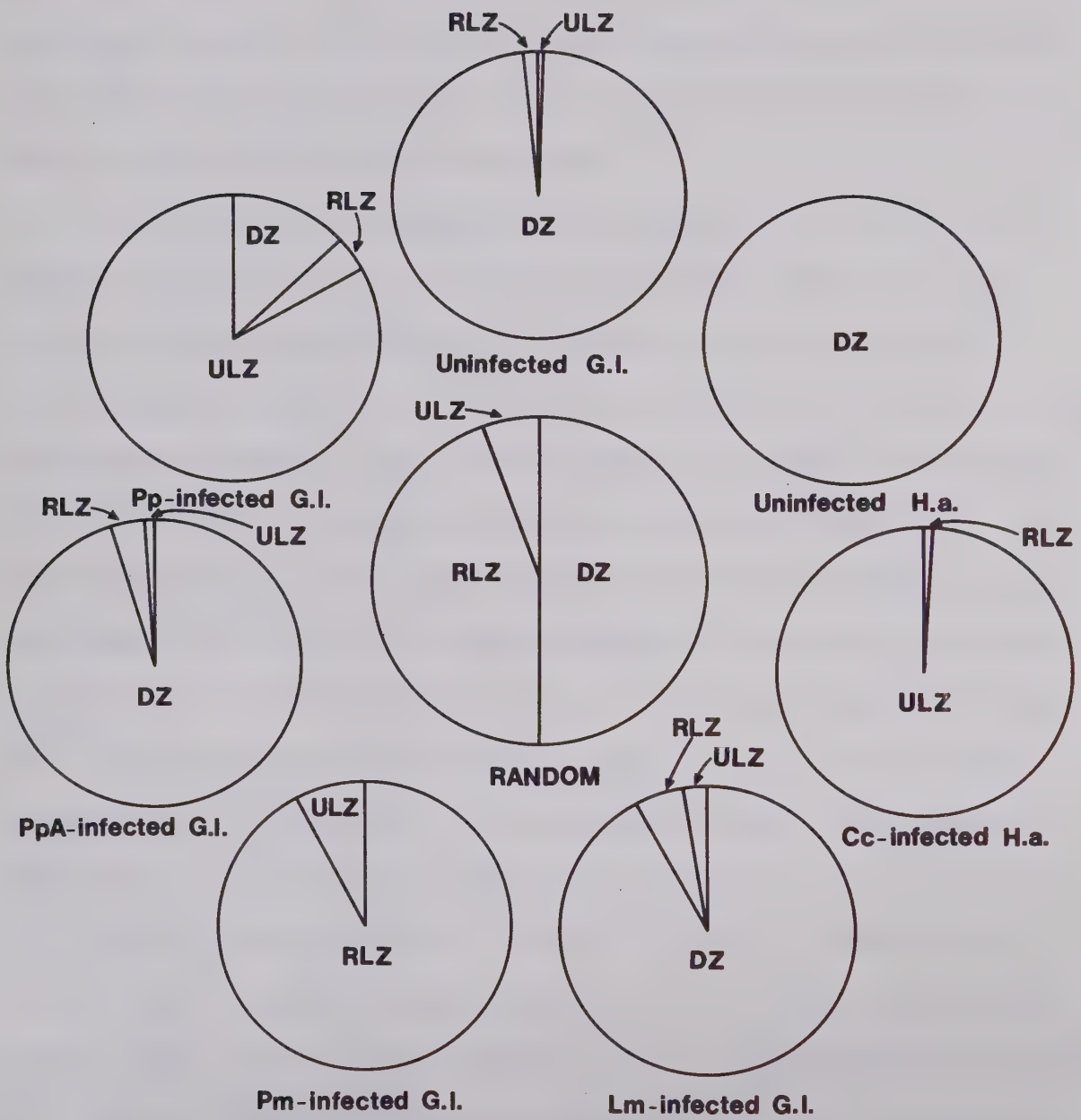
	Analysis of Variance		Multiple Range Test (mean arcsine transforms)					
	F	P	Un.**	*P.p.*A**	*L.m.***	*P.m.***	*P.p.***	*C.c.***
ULZ* (with *C.c.*** included)	144.44	<.001	2	4	8	16	61	88
ULZ* (*C.c.* not included)	52.75	<.001	2	4	8	16	61	
TLZ*	104.25	<.001	4	6	16	74	90	

Means not underscored by the same line are significantly different at the 5% level.

*ULZ = Upper 3 cm of Light Zone; TLZ = Total Light Zone.

**Un. = Uninfected *Gammarus lacustris*; *P.p.*A = Infected with *Polymorphus paradoxus* acanthellae; *L.m.* = Infected with *Lateriporus mathevossianae*; *P.m.* = Infected with *P. marilis*; *P.p.* = Infected with *P. paradoxus*; *C.c.* = *Hyalella azteca* infected with *Corynosoma constrictum*.

Fig. 6. Proportion of time spent by infected and uninfected amphipods in different areas of a light-dark choice aquarium. ULZ, upper 3 cm of light zone; RLZ, remainder of light zone; DZ, dark zone; *G.l.*, *Gammarus lacustris*; *H.a.*, *Hyalella azteca*; *P.p.*, *Polymorphus paradoxus*; *P.p.*A, *P. paradoxus* acanthellae; *P.m.*, *P. marilis*; *L.m.*, *Lateriporus mathevossianae*; *C.c.*, *Corynosoma constrictum*. Random values calculated from relative volume of each zone.


RLZ
ULZ
DZ
Uninfected G.I.
RLZ
DZ
ULZ
DZ
Uninfected H.a.
ULZ
RLZ
DZ
RANDOM
RLZ
Pp-infected G.I.
ULZ
DZ
PpA-infected G.I.
RLZ
ULZ
Cc-infected H.a.
ULZ
RLZ
Pm-infected G.I.
RLZ
ULZ
DZ
Lm-infected G.I.

The uninfected amphipods and those infected with *P.p.*A and *L.m.* all appeared to be strongly photophobic. The few which entered the lighted zone were there for very short intervals and, while there, were randomly distributed between the ULZ and RLZ. The uninfected hyalellids were never recorded in the lighted zone, although some were seen at the very edge of the dark zone. At the end of the observations, most were recovered from the mud of the dark zone.

Conversely, the amphipods infected with *P.p.*, *P.m.*, and *C.c.* showed a distinct preference for the lighted zone. Within that zone, the *P.m.*-infected gammarids were distributed at random. The *P.p.*-infected gammarids showed a strong preference for the ULZ (Table 4). One *P.p.*-infected gammarid spent only 20 minutes, 32 seconds, in the lighted zone, considerably less than the others in its group (Appendix 1). The arcsine transforms of the time it spent in the ULZ and TLZ were compared with those of the rest of the group and those of controls by the method of Sokal and Rohlf (1969:223-226); they were not significantly different ($p > .3$) from those of the rest of its group, but were significantly greater ($p < .01$) than those of the uninfected group. The hyalellids infected with *C.c.* showed an even stronger preference for the ULZ than *P.p.*-infected gammarids (Table 4 and Fig. 6). All 12 remained in the lighted zone throughout the observations, and only 2 of these left the ULZ, and then only for short intervals. The *C.c.*-infected hyalellids and *P.p.*-infected gammarids were usually associated with the floating wood and vegetation in the ULZ. The association was a loose one, and different from that of the "clinging" seen in *P.p.*-infected gammarids in the field.

RESPONSES TO A HORIZONTAL GRADIENT OF ILLUMINATION

In the light-dark zone tests, the amphipods were exposed to a sharp contrast in illumination with a very narrow intermediate, or twilight, zone. The illumination gradient cylinder, described under General Methods (Fig. 4), provided a gradient of illumination in which the amphipods could be tested for a preference for an intermediate level of illumination. Uninfected gammarids and ones infected with *P.p.* and *P.m.* were the only amphipods used in the tests; hyalellids could not be observed satisfactorily due to their size and color.

The gammarids (in groups of 3-5) were transferred to the darkroom in a funnel and released via the funnel into the cylinder through the opening in section 10. After a 5-minute period, the projector lamp was switched on; the position of each visible gammarid was recorded every 30 seconds thereafter for 10 minutes. Any gammarids not visible were considered to be somewhere in the dark zone.

Results

There were no indications of a preference for any intermediate level of illumination during any of the testing. The uninfected gammarids congregated in the dark zone, the infected gammarids in the brightest zone.

Most (18/25) of the uninfected gammarids remained in the dark zone throughout the observations. Only four entered section 1, the area with the highest level of illumination (Table 5), only for very brief periods (30 seconds or less), and only in the last half of the test.

The *P.m.*-infected gammarids were very active in the cylinder throughout the tests. Most moved to section 1 early in the tests, but

Table 5. Movements of infected and uninfected *Gammarus lacustris* into and out of the brightest section of a horizontal gradient of illumination

	No. tested	No. arriving	No. staying	No. leaving	No. returning
Uninfected	25	4	0	4	1
Polymorphus paradoxus	25	21	15	6	2
P. marilis	21	19	4	15	8

Table 6. The distribution of infected and uninfected *Gammarus lacustris* in a horizontal gradient of illumination

	No. of observations	No. of observations		
	No. of observations	Bright Zone	Intermediate Zone	Dark Zone
Uninfected	500	14	42	444
Polymorphus marilis	420	238	70	112
P. paradoxus	500	343	55	102

few remained there (Table 5); instead, they moved irregularly, usually within the bright and intermediate zones. The overall number of times they were recorded in the bright zone (Table 6) was significantly greater (2 x 3 Chi-square = 400, $p < .001$) than those of the uninfected group, but significantly less (Chi-square = 14.4, $p < .001$) than those of the *P.p.*-infected gammarids.

The *P.p.*-infected gammarids also moved into section 1 early in the tests, the majority by 2 minutes. The median time taken to reach section 1 was not significantly less than that of *P.m.*-infected gammarids. However, a high proportion stayed in that area for the remainder of the observations (Table 5), typically circling around the periphery of the cylinder with their dorsal sides placed against the glass adjacent to the light source. As a consequence, the number of bright zone recordings (Table 6) was significantly higher than those of the *P.m.*-infected gammarids (Chi-square = 14.4, $p < .001$) as well as those of the uninfected gammarids. Four *P.p.*-infected gammarids which did not emerge from the dark zone were found at the end of the test, clinging near where they were introduced.

RESPONSES TO BOTTOM LIGHT

The behaviour of the *P.p.*-infected gammarids and *C.c.*-infected hyalellids during the light-dark zone tests can be interpreted as expressions of a positive phototaxis or a negative geotaxis. In order to distinguish between the influence of light and gravity on their responses, infected and uninfected amphipods were exposed to two series of bottom light experiments. In one series, they were exposed for various time periods to light directed from the bottom of the modified light-dark

choice aquarium. Up to ten gammarids infected with *P.p.* or hyalellids infected with *C.c.*, plus equal numbers of uninfected controls, were introduced into the aquarium in complete darkness and allowed 10 minutes to settle; the bottom light was then turned on and their immediate responses were recorded. They were then left undisturbed in the aquarium for 15-minute, 30-minute, or 1-hour periods. At the end of the periods, the numbers and positions of infected and uninfected amphipods in the light zone were recorded, then the two zones were sealed off by a plexiglass partition inserted into grooves cut in the frame of the aquarium and both zones searched for any additional amphipods.

In the other series, the direction of the light was alternated between sources at the bottom and top of the aquarium. Uninfected gammarids, and those infected with *P.p.*, *P.p.*A, *P.m.*, *L.m.*, and *L.* spp., plus uninfected hyalellids, and those infected with *C.c.*, were tested. The amphipods were introduced singly (for smaller, less conspicuous individuals), or in one or two pairs (one infected and one uninfected), into the aquarium in complete darkness, and allowed 3-5 minutes to settle. The light was switched on at the bottom and the immediate responses of the amphipods were recorded. The light was then alternated between the two sources, and the responses of the amphipods to each change in light direction were recorded.

Both experiments are typical of those used for demonstrating the dorsal light reaction in aquatic invertebrates (see Fraenkel and Gunn 1961). The reaction, although well defined for many invertebrates (see Fraenkel and Gunn 1961), has not been described for *G. lacustris* and *H. azteca*. Therefore, the dorsal-ventral orientation of the amphipods and

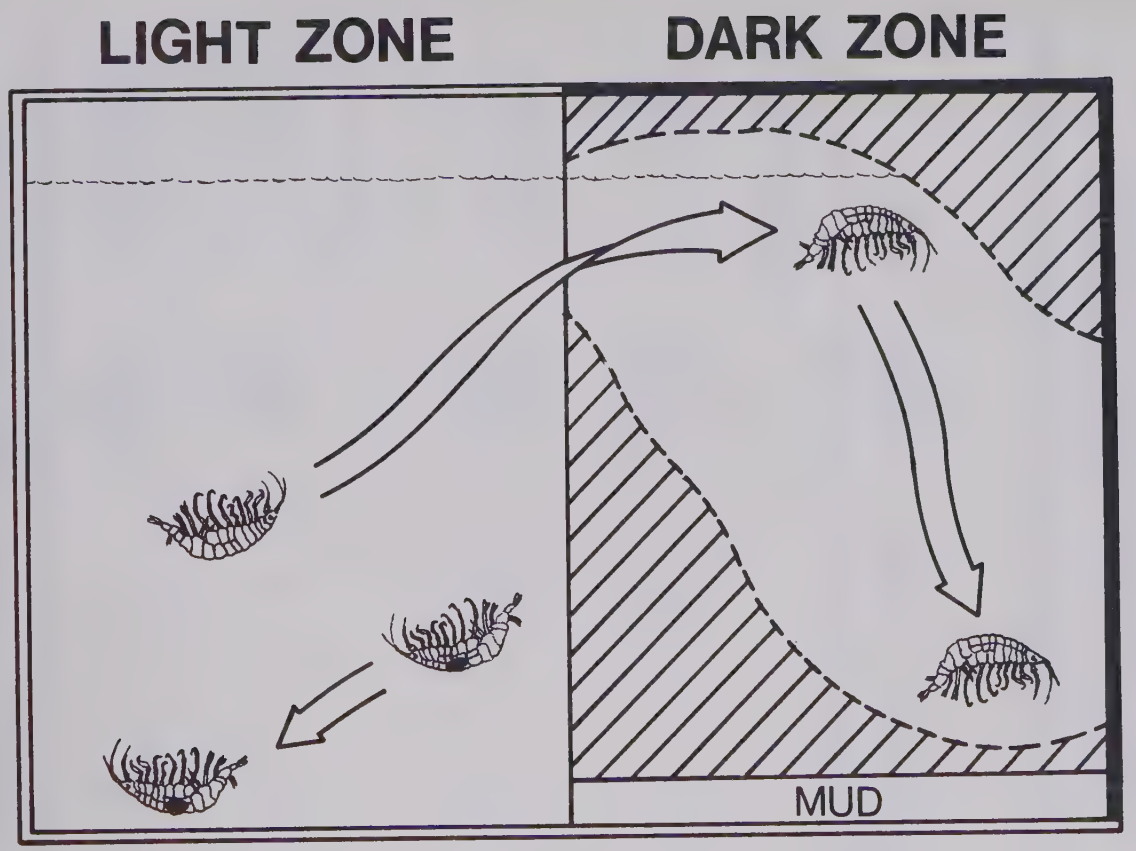
the manner in which they made any correction in their orientation were also noted.

Results

The initial responses of all the amphipods during the bottom light-dark zone tests were difficult to record due to the numbers observed concurrently. However, there were obvious differences in the responses of infected and uninfected individuals caught in the light zone when the tests began. The uninfected amphipods immediately oriented their dorsal sides toward the light source as in a typical dorsal light response, gammarids by rolling to one side and hyalellids usually by a backward somersault, and swam diagonally upwards into the dark zone. On reaching the dark zone, they reoriented, using the same maneuver, to their normal position with dorsal side uppermost, and swam down to the mud at the bottom (Fig. 7). Amphipods infected with *P.p.* or *C.c.* showed the same dorsal-positive orientation to the bottom light, but swam toward it, and congregated in the area of most intense light (i.e., the bottom glass adjacent to the light source) with their dorsal sides pressed against the glass (Fig. 7). All of the infected hyalellids and the majority (89%) of the *P.p.*-infected gammarids were in this position whenever checked (Table 7). Infected gammarids were occasionally found swimming, dorsal side down, in the remainder of the light zone, but no association with the water surface or preference for the upper part of the zone was evident.

The partition which was used to seal off the two zones caught one uninfected gammarid on the bottom at the boundary, but on the light zone side. This was the only uninfected amphipod found in the bottom light

Fig. 7. Responses of an uninfected *Gammarus lacustris* and one infected with *Polymorphus paradoxus* (represented by black spot) in the bottom light-dark zone aquarium.


LIGHT ZONE
DARK ZONE
MUD

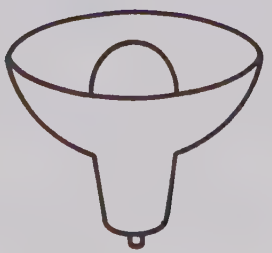


Table 7. Distribution after various intervals of infected and uninfected amphipods in different areas of a light-dark choice aquarium with the light directed from the bottom

	Time interval	No. tested	No. of observations	Distribution		
				BLZ*	RLZ*	DZ*
Gammarus lacustris						
Uninfected	1 hr	12	1	0	1	11
	30 min	32	1	1	4	27
	15 min	20	4	0	0	80
Polymorphus paradoxus	1 hr	12	1	12	0	0
	30 min	32	30	30	0	2
	15 min	20	4	61	7	12
Hyalella azteca						
Uninfected	1 hr	2	1	0	0	2
	30 min	7	1	0	0	7
Corynosoma constrictum	1 hr	2	1	2	0	0
	30 min	7	1	7	0	0

*BLZ = Bottom of Light Zone; RLZ = Remainder of Light Zone; DZ = Dark Zone.

zone; the others seen in the lighted zone were at the surface, swimming or associated with the floatage or the corners of the aquarium. Uninfected gammarids were sometimes seen along the bottom, at the edge of the dark zone where the thinner mud allowed a limited amount of light to penetrate. They quickly retreated if their activity carried them into more intense light. No uninfected hyalellids were found in the light zone during the observations (Table 7).

A larger number of infected and uninfected amphipods were tested by the alternating light system. All showed the dorsal orientation to light, and their respective phototactic responses were consistent with those seen in the bottom light-dark zone tests.

The uninfected amphipods and ones infected with *P.p.*A, *P.m.*, *L.m.*, and *L.* spp. showed a strong negative phototaxis throughout the tests (Table 8). In their first response to the bottom light, the gammarids swam, at a normal speed, toward the water surface at the corners of the aquarium, and remained there until the light sources were switched, after which they swam to the bottom corners. The corners were the furthest points from the light source and possibly the areas with the lowest level of illumination.

Conversely, the majority (80%) of the gammarids infected with *P.p.* were positively phototactic. The individuals which showed a positive response to the bottom light all swam, dorsal side down, to the area nearest the light source where they remained with their dorsal sides against the glass. They often swam in small circles against the glass while in this position. Those which were at the bottom when the bottom light was turned on responded by rolling over to the dorsal side. When

Table 8. Phototactic responses of infected and uninfected amphipods in an alternating light system

	No. tested	Phototaxis		
		+	0	-
Gammarus lacustris				
Uninfected	279	0	0	279
Polymorphus paradoxus cystacanths	155	124	8	23
P. paradoxus acanthellae	50	0	0	50
P. marilis	43	0	0	43
Lateriporus mathevossianae	25	0	0	25
Lateriporus spp.	20	0	0	20
Hyalella azteca				
Uninfected	140	0	0	140
Corynosoma constrictum	117	77	6	34

responding positively to the top light, the gammarids appeared to dig at the air-water interface, a motion similar in appearance, but less pronounced than in the skimming behaviour.

About one-fifth of the *P.p.*-infected gammarids did not respond positively to light. Of these, 8 corrected their orientation, moved slightly, then settled to the bottom, regardless of the direction of the light. These are shown in Table 8 as having no phototactic response. The other 23 infected gammarids showed a negative phototaxis, but swam away from the light at a slower speed than was characteristic of uninfected gammarids.

About two-thirds of the *C.c.*-infected hyalellids were positively phototactic. Their response to the bottom light was identical to that of the positively phototactic *P.p.*-infected gammarids. When the top light was on, they swam up to the surface, ceased all movement and settled to the bottom, then swam up to the surface again. They appeared to be more sluggish in their movements than uninfected hyalellids, and the settling appeared to be due to fatigue.

About one-fifth of the infected hyalellids were obviously negatively phototactic; the rest were inconsistent in their responses, and the division between no phototaxis and a negative phototaxis in Table 8 is based on the overall balance of their responses in a series of reversals of the direction of the light.

RESPONSES TO DISTURBANCE

The uninfected amphipods and the few gammarids infected with *P.m.* and *L.* spp. which were encountered at the water surface in the study lakes quickly evaded any disturbance by diving to the bottom. The *P.p.*-infected

gammarids never dove when they were disturbed, but exhibited skimming and/or clinging behaviour, suggesting that these phenomena may be expressions of an abnormal evasive response.

Tests and observations on the evasive behaviour of infected and uninfected amphipods were continued in the laboratory under "normal aquarium" and experimental conditions. The "normal aquaria" were laboratory aquaria in which amphipods were held after being collected in the study lakes (see General Methods for details). Whenever amphipods were sighted at the surface of these aquaria, they were disturbed directly (by touching the amphipod) or indirectly (by disturbing the water or the material on which it was located), and their responses recorded.

In order to quantify the proportions of the populations found at the surface and their responses to disturbance, four comparatively large samples of uninfected and *P.p.*-infected gammarids (up to 175 each) collected from Cooking Lake were placed in an 80-gallon laboratory aquarium. The numbers of gammarids clinging to three types of floatage (wooden sticks, reeds, and *Potomogeton* sp.) and to the corners of the aquarium (in the upper 3 cm of water) were recorded three times daily, in the morning, at noon, and in the mid-afternoon. The floatage was picked out of the water and the clinging gammarids were identified and counted. The responses (such as diving or skimming) of gammarids which did not cling, yet were associated with the floatage before it was disturbed, were followed as closely as possible. Preliminary observations indicated that the disturbance in the water was enough to test the responses of gammarids in the corners.

The experimental manipulations were designed to test the influence

of the phototactic responses of infected and uninfected amphipods on their evasive responses. The tests were performed in conjunction with the alternating light experiments. After recording the initial responses, the same amphipod was disturbed, by touching it with a glass rod, and its responses to alternating sequences of light from the bottom and top of the aquarium were recorded. (The first 20 *P.p.*-infected *G. lacustris* and the first 17 *C.c.*-infected *H. azteca* which were tested in the alternating light system were not included in these disturbance tests.) Amphipods which exhibited a positive phototaxis were tested with an additional light directed from the side of the aquarium.

Uninfected and *P.p.*-infected gammarids were subjected to additional experiments. In one series, groups of five infected and uninfected gammarids were placed in the aquarium in the dark. They were then disturbed by gently stirring the water with a small wire net. The overhead light of the room was switched on and the vertical position (top, middle, bottom) of each gammarid was recorded as quickly as possible. As a control, the same test was performed under an overhead light.

In a third series, the gammarids (up to 20 each) were placed in water which had a low dissolved oxygen content--3-4 ppm (19 C) as determined by Burke's (1962) micro-Winkler test. The top light was switched on and the gammarids were left undisturbed in the water until they showed signs of oxygen deprivation (2-4 hours), by congregating at the surface along the sides of the aquarium. The dissolved oxygen at this time was too low to be measured accurately by the micro-Winkler method, but the tests did indicate that it was less than .2 ppm. The gammarids were then disturbed and their responses were recorded. In the initial test, 50 infected and 50 uninfected gammarids were tested with the top light

only; in a subsequent test, 25 of each were tested with top and bottom light sources.

Results

Uninfected amphipods, and those infected with helminths other than *P.p.* and *C.c.*, were rarely found at the surface. In order to test their evasive responses, they were therefore baited to the surface with pieces of reeds and lettuce leaves, as the only food supplied. The responses of 42 gammarids infected with *P.m.*, 30 with *P.c.*, 30 with *L.m.*, and 13 with *L.* spp., were recorded after they had been disturbed at the surface. Their responses were identical to those of numerous uninfected amphipods which were tested. All immediately dove to the bottoms of the aquaria, and many burrowed into the mud, particularly after they had been disturbed directly.

Hyalellids infected with *C.c.* were frequently found at the surface; the majority were caught in the surface tension, apparently unable to free themselves, and many were dead or moribund. Eight of the 30 infected hyalellids tested did not dive at all, even after being disturbed repeatedly, and continually returned to the surface after being pushed several centimeters below it. The other infected hyalellids were slower in their responses than uninfected ones, and many of the former returned to the surface within one or two minutes. They showed no skimming or clinging behaviour.

As in the field observations, *P.p.*-infected gammarids were encountered at the surface much more frequently than were uninfected amphipods; they all exhibited the skimming and/or clinging behaviour when disturbed. The *P.p.*-infected gammarids appeared to show a higher

sensitivity to disturbance than any of the other amphipods tested. I have occasionally observed skimming and clinging to be initiated by slight, probably commonplace, disturbances such as collisions with other gammarids or air bubbles or when the aquarium was jarred slightly. Infected gammarids were often seen rising to the surface and skimming when no apparent disturbance had taken place. Uninfected amphipods, or those infected with other helminths, do not respond so strongly to slight disturbances. They will usually move from the upper to the under surfaces of floating material or swim slowly down to a lower level (but not to the bottom). Only when directly disturbed will they dive to the bottom and burrow.

In the quantification studies, some uninfected gammarids were associated with the sticks and reeds before the material was disturbed, but their numbers could not be accurately determined because they dove to the bottom as soon as the articles were touched. None of the *P.p.*-infected gammarids dove, the few which did not cling immediately to the material with which they were associated skimmed along the water surface and eventually clung to the corners of the aquarium, often crawling as much as 2-3 cm above the water. The majority (61% of those clinging) were found on the reeds (Table 9), sometimes numbering up to 40 on one reed. Overall, somewhat over half of the infected gammarids were associated with floating material. However, the numbers at the surface in the afternoon were significantly greater ($t = 3.13$, $p < .05$) than in the morning. The midday counts were intermediate, suggesting a gradual increase during the day in the proportion of *P.p.*-infected gammarids associated with the surface material.

Table 9. Numbers of *Gammarus lacustris* infected with cystacanths of *Polymorphus paradoxus* clinging to various substrates at different times of the day

Test	Sample size	Time	No. clinging				
			Reeds	Sticks	Corners	*Potomogeton** spp.	Total
1	60						
		1000	14	2	0	-	16
		1200	21	5	6	-	32
		1600	30	4	2	-	36
2	50						
		0900	18	0	0	-	18
		1200	11	12	0	-	23
		1500	19	9	5	-	33
3	175						
		0900	10	34	33	-	77
		1200	71	2	5	6	84
		1500	50	4	30	12	96
4	175						
		1000	61	15	18	3	97
		1200	74	9	29	4	116
		1600	84	5	35	2	126
Total	460						

*Used only in tests 3 and 4.

When the uninfected amphipods and gammarids infected with *P.p.*A, *P.m.*, *L.m.*, and *L.* spp. were disturbed in the alternating light aquarium their negatively phototactic responses were greatly intensified; they swam more rapidly and directly away from the light. After responding in this manner to the bottom light, they "dug" rapidly at the water surface with their gnathopods, a motion very similar to the skimming behaviour of *P.p.*-infected gammarids. When the top light was used, the amphipods darted back to the bottom and dug at the glass; some crawled under or covered themselves with the small amount of debris which had collected on the bottom.

The proportion of the *P.p.*-infected gammarids that showed a positive phototaxis was significantly increased after disturbance (Table 10). All but seven showed a positive reaction, which was more intense than before being disturbed. Their movements were similar to those of uninfected gammarids, but opposite with respect to light direction. When the light was directed from the bottom or side of the aquarium, they instantly swam to it. The dorsal orientation against the glass was not as evident as in their responses before disturbance due to a rapid circling, produced by the same sort of digging motion displayed by uninfected gammarids. When responding in light directed from above, the digging movements produced the skimming behaviour. During some of the tests, pieces of wood were placed at the top and bottom of the aquarium. After *P.p.*-infected gammarids had responded phototactically to the disturbance, they readily clung onto the wood (at the top or the bottom of the aquarium) if they came in contact with it. The clinging gammarids did not respond to any subsequent changes in light direction until they were forcibly removed from the wood.

Table 10. Phototactic responses of infected amphipods, before and after being disturbed, in an alternating light system

	Phototaxis before disturbance			Phototaxis after disturbance			
	+	0	-	+	0	-	P*
Gammarus lacustris + *Polymorphus paradoxus*	104	8	23	128	0	7	<.001
Hyalella azteca + *Corynosoma constrictum*	60	6	34	41	0	59	<.05

*Chi-square comparison of positive and combined "0" and negative responses before and after disturbance.

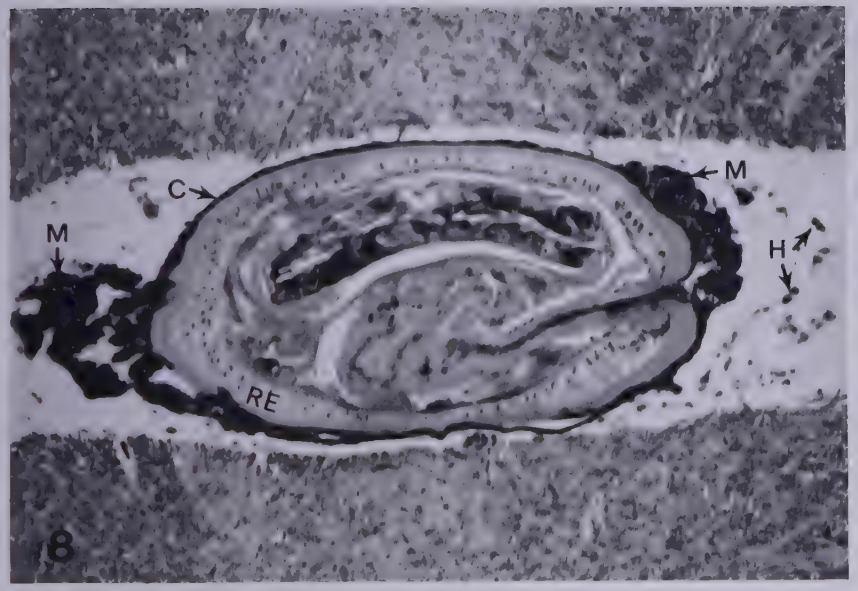
An examination of the cystacanths from the seven *P.p.*-infected gammarids which showed negative phototactic responses to disturbance revealed that only two were apparently normal. Two others were actually in the final stages of the invagination process (and therefore transforming from acanthellae to young cystacanths). The remaining three were missing the thin connective tissue envelope which normally surrounds the cystacanths (Fig. 9). All three had dark pigments concentrated at the sites of fore- and hind-body invagination. Histological examination of these cystacanths showed that they were encapsulated by host haemocytes (of at least 4 types, as shown in Fig. 11) and that the dark pigments were melanin deposits within the capsule (Fig. 8). Melanin was also found in the invaginated portions of two of the specimens (Fig. 10). There were several layers of haemocytes in the areas of melaninization; the capsule was otherwise one cell layer thick.

The oxygen content of the water had an obvious effect on the behaviour of uninfected and *P.p.*-infected gammarids. Before being disturbed, all of the gammarids remained at the water surface whether the light was being directed from below or above. However, when disturbed, they responded as they did under aerated conditions, i.e., the infected were positively and uninfected negatively phototactic. Both infected and uninfected gammarids returned to the surface 3-5 minutes after the disturbance, regardless of the direction of the light.

In 10 observations on the positions of gammarids after they had been disturbed in the dark, all 50 uninfected, and 44 *P.p.*-infected gammarids were found on or near the bottom of the aquarium. Six infected individuals were found clinging in the corners at the water surface. When the same experiment was run under a normal overhead light, only 5

Figs. 8-10. Longitudinal sections of cystacanths of *Polymorphus paradoxus*. Embedded in sheep brain tissue. Stained with H and E. Fig. 8. Cystacanth which has been encapsulated by host haemocytes. Haemocytes (H) and melanin deposits (M) are concentrated at the anterior and posterior ends of the cystacanth. Fig. 9. Normal cystacanth with the surrounding envelope of connective tissue (E). Fig. 10. Anterior end of a cystacanth showing haemocytes (H) and melanin deposits (M) in the invaginated portion of the fore-body. C, capsule of haemocytes; RE, radially striated epidermis of cystacanth; Cu, cuticle of cystacanth.

Fig. 11. Section through the periphery of the haemocytic encapsulation showing different types of associated haemocytes.


C
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M
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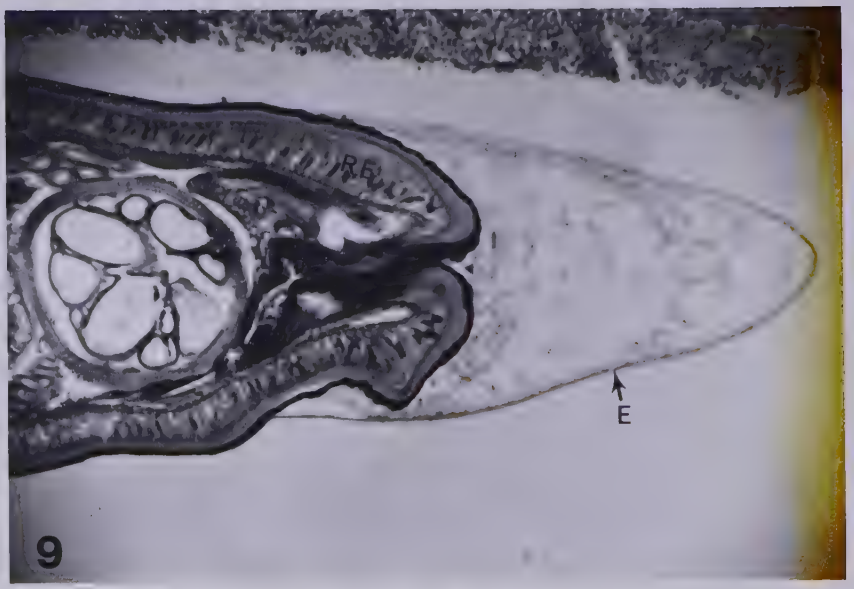
RE
8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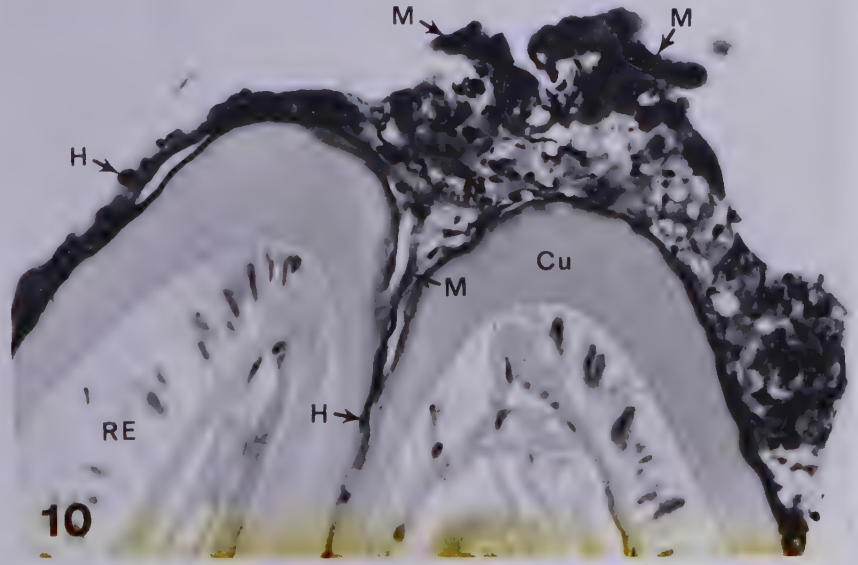
M
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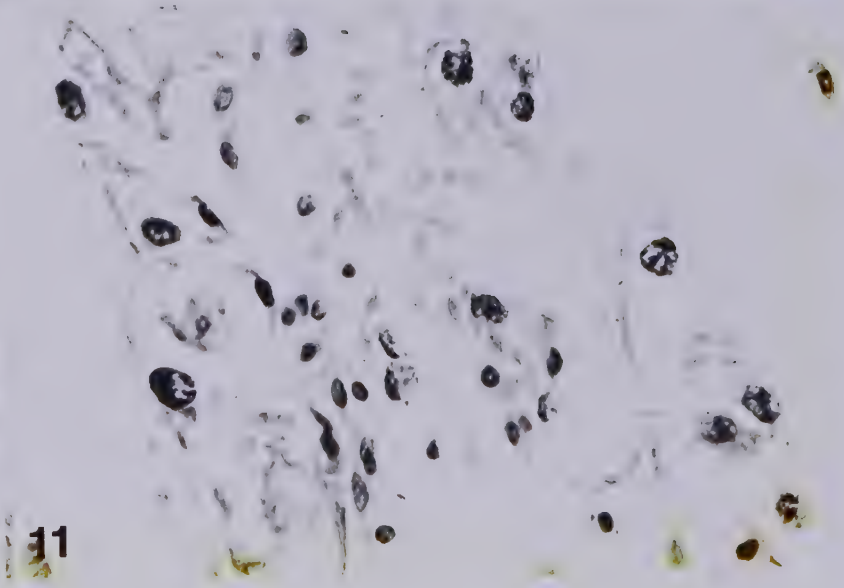
11

infected gammarids were on the bottom, 11 were skimming when counted, and the other 34 were clinging in the corners or on the air hose.

After they were disturbed, the phototactic responses of *C.c.*-infected hyalellids were either strongly negative or strongly positive; none showed inconsistent responses, as some did before being disturbed. However, the proportion which exhibited a positive phototaxis was significantly lower than prior to disturbance (Table10). The behaviour of the positively phototactic individuals was identical to that observed during previous tests. The infected hyalellids did not show the extreme sensitivity to disturbance exhibited by *P.p.*-infected gammarids, and their positive responses to light were much slower.

DEVELOPMENT OF THE ALTERED EVASIVE RESPONSE OF *GAMMARUS LACUSTRIS* INFECTED WITH *POLYMORPHUS PARADOXUS*

The tests described above established rather conclusively that gammarids infected with *P.p.* cystacanths display altered photophilic, phototactic and evasive responses not present in gammarids infected with *P.p.* acanthellae. In order to determine the stage of development of the acanthocephalan at which the altered evasive behaviour appears, infected gammarids were tested at intervals throughout the development from the acanthella to the cystacanth. Gammarids with very conspicuous acanthellae were selected (so that the development could be followed *in vivo*) and transferred to a special maintenance aquarium. Larvae were considered to be at the cystacanth stage when the fore- and hind-body of the acanthella was fully invaginated, as in Fig. 8; in experimental infections, the process of invagination takes 25-27 days and is completed 40-42 days after infection (at 19 C).

Infected gammarids were tested once weekly during the initial stages of invagination (acanthella), twice weekly towards the end of the process (late acanthella), and daily after the invagination was complete (cystacanth). The gammarids were tested for their response to disturbance in the maintenance aquarium. Any gammarid which did not dive immediately after being disturbed was transferred to the darkroom and tested for its response to disturbance in the alternating light system. Once an altered response was apparent, it was retested daily thereafter for a period of two weeks.

Results

Gammarids containing young cystacanths (1-10 days after invagination) invariably dove when disturbed; 39 were tested in the alternating light system and were strongly negatively phototactic after being disturbed.

Approximately 10-15 days after invagination, some infected gammarids did not dive immediately, but swam at the surface to the corner before diving. When tested with the alternating light system, they were strongly negatively phototactic. On two occasions, involving 2 and 3 gammarids harbouring 10 day-old cystacanths, their activity more closely resembled, but was not identical to, the skimming behaviour. When tested with the alternating light system, these gammarids showed both negative and positive responses; there was no evidence of the clinging response. There was no further change in their behaviour until the fully-developed evasive alterations were evident on the 17th or 18th day.

Fully developed skimming and clinging responses appeared suddenly (within 24-25 hours of the previous test) 15-20 (mean, 17) days after

Table 11. First appearance of altered evasive response in *Gammarus lacustris* parasitized by larvae of *Polymorphus paradoxus*

Days after formation of cystacanth	Natural infections	Experimental infections
15	2	3
16	6	4
17	12	11
18	3	4
19	2	1
20	0	2
Total	25	25
Mean	16.9	17.1

the formation of the cystacanths (Table 11). The reversed phototactic response was also evident at this time. These gammarids were also more sensitive to disturbance; this sensitivity was magnified during the following two weeks. Once the altered evasive response appeared, it was consistently present in subsequent tests.

THE INFECTIVITY OF *POLYMORPHUS PARADOXUS* CYSTACANTHS

If the behavioural alterations of gammarids infected with *P.p.* are considered as manifestations of an evolutionary strategy adopted by the parasite for enhancing their transmission to the definitive host, then the timing of the onset of the behavioural changes relative to the infectivity of the cystacanths is extremely important. Most acanthocephalans are infective to their definitive hosts as soon as the cystacanths are formed, but there are exceptions, such as *Polymorphus trochus*, which requires an additional two weeks after the cystacanth is fully formed (Podesta and Holmes 1970) and *Prosthorhynchus formosus*, which also has a long period of maturation as a cystacanth before becoming infective (Schmidt and Olsen 1964).

Unfortunately, a study on the day by day development of the infectivity of *P.p.* cystacanths could not be done, due to the difficulty of obtaining a sufficient number of young cystacanths. It was possible, however, to compare the infectivity of cystacanths from gammarids which were exhibiting the altered behaviour with that of young, 1-10 day, cystacanths from gammarids showing apparently normal behaviour.

The criterion used to compare and evaluate the infectivity of cystacanths was the proportion of the challenge infection recovered from

the intestine of a hamster (shown in previous experiments to be a suitable experimental host) 5 days after exposure.

The infectivity of young (1-10 day) cystacanths was tested whenever five or more became available. An equal number of cystacanths from gammarids which were exhibiting the altered behaviour were tested concurrently, using another hamster of the same brood. The hamsters were from either the LHC or LSH strains and were 1-4 weeks old when used. They were lightly anaesthetized and force fed gelatin capsules containing the cystacanths in .5 cc of dechlorinated water. At necropsy 5 days later, the hamsters were opened, the mesenteries and periphery of the intestine and other visceral organs were examined for encapsulated worms, and the small and large intestines were removed and examined.

Results

In a preliminary test, the infectivity of known-age cystacanths raised in gammarids infected in the laboratory was determined. A hamster was fed 10 young cystacanths (1-7 days post-invagination), from gammarids showing normal behaviour; 2 weeks later, another hamster was fed 10 cystacanths (17-18 days post-invagination) from gammarids in which the altered behaviour had just become apparent. Each hamster was examined 20 days after infection. The former was negative, the latter contained two ovigerous females, plus two males encapsulated in the mesenteries.

In all other infectivity experiments (Table 12), the cystacanths were obtained from natural infections. Thus, the derivation of these cystacanths was not known, nor was the age of cystacanths which had been obtained from gammarids exhibiting the altered behaviour. The young

Table 12. Relationship between the infectivity of *Polymorphus paradoxus* cystacanths to hamsters and the altered behaviour in *Gammarus lacustris*

	Older cystacanths (*G. lacustris* showing behavioural alterations)		Young (1-10 day) cystacanths (*G. lacustris* showing normal behaviour)	
Test	No. administered	No. recovered after 5 days	No. administered	No. recovered after 5 days
1	5	3	5	0
2	10	4 + 1 encapsulated in mesentery	10	0
3	5	5	5	0
4	9	3	9	0
5	10	2 + 2 encapsulated	10	1
Total	39	17 + 3 encapsulated	39	1

cystacanths, however, were all known to be 1-7 days old with the exception of two cystacanths used in experiment #5 which were 8 and 10 days old.

About half of the cystacanths from gammarids showing altered behaviour were recovered from the intestine of hamsters as young adults, which had undergone standard growth and development for a 5-day post-infection period. The only exceptions were those specimens encapsulated in the mesenteries, which were smaller. Such encapsulated specimens are usually found in naturally infected muskrats.

Only one of the 39 young cystacanths from gammarids not showing altered behaviour became established; it was from the hamster given the 8 and 10 day old cystacanths.

DISCUSSION

Behaviour and Distribution

The observations presented in this study illustrate that light has an extremely important influence on the behaviour and distribution of *G. lacustris* and *H. azteca*. The uninfected amphipods were strongly photophobic and consistently negatively phototactic regardless of the direction of light. The dorsal light reaction was well developed in all the amphipods, which shows that, when present, light is clearly the dominating environmental factor (stimulus) in influencing their body orientation.

The dorsal light reaction has been demonstrated in several species of crustaceans which lack statocysts or statocyst-like systems; in those species with well developed statocysts, the reaction is exhibited only after the statocysts have been removed (Carthy 1958, Fraenkel and Gunn

1961, Marler and Hamilton 1966). *Gammarus locusta* has well developed statocyst-like organs (frontal organs) and does not exhibit the dorsal light reaction, whereas the reaction is manifested by *G. pulex*, which has poorly developed organs (Langenbuck 1928). Schmitz (1967) did not include receptors in his study of the anatomy of *G. lacustris*, but he has observed statocyst-like structures in both *G. lacustris* and *H. azteca* (personal communication). In these two species, these receptors appear to be overridden in the presence of light, but may serve to orient the amphipods in the absence of light, and were probably responsible for the reorientation of uninfected amphipods when they entered the dark zone during the bottom light tests.

Another important factor in affecting the overall distribution of the amphipods is the kind of food which is available. Both species of amphipod are omnivorous (Pennak 1953, Mennon 1966, Hargrave 1970, this study) and utilize the most available food. In the study lakes this consists mainly of benthic organisms, such as diatoms and chironomid larvae, and organic detritus. Food items are seldom available at the water surface of the lakes, although duck weed (*Lemna minor*) is common in some areas of Hastings Lake. Floating organic matter, such as pieces of reeds or dead waterfowl, are occasionally available and are utilized by the amphipods, but not extensively.

In laboratory aquaria the amphipods appeared to restrict their foraging and feeding activities to the bottom mud surface. They were immediately attracted to food such as yeast or recently killed leeches or insect larvae. When the food there was depleted, however, they foraged throughout the entire aquarium. The foraging behaviour of *G. lacustris*

near the water surface is very distinctive. The gammarids swim in a roller-coaster pattern just below the surface, often turning onto their sides and grasping at the surface, or at floating material. After capturing a food item at the surface, they returned to the bottom to feed. The gammarids were never immediately attracted to the floating plant materials used to "lure" them to the surface, and fed on the latter only when other items were not available.

The extremely photophobic behaviour displayed by uninfected amphipods during laboratory tests may be due partly to the relatively high light intensities which were used. In the study lakes, the amphipods can easily avoid light by diving into deeper water, because of the rapid extinction of light in the turbid water. Photometer readings, using a Gemware submarine photometer (model #268-WA-310), in Cooking Lake on a clear day in late June, 1970, showed a 77% reduction in light penetration at 25 cm; no penetration could be detected at 45 cm. The turbidity of this lake is remarkably consistent from June to October. Hastings Lake is less turbid, but the extensive algal populations during the summer also reduce light penetration; it also has much more vegetative cover in the shallow areas where the amphipods are concentrated. Since Phipps (1915) found that a long period (several weeks) of captivity in the laboratory "had an effect in lessening the negative responses of the amphipods to intensity and to direction of (light) rays," several measures (see General Methods, p. 11) were taken to prevent habituation of the amphipods to more intense light while in laboratory aquaria.

Mennon (1966, 1969) studied the distribution of *G. lacustris* in Big Island Lake, similar to and only 5 miles west of Cooking Lake. Among

other observations, he studied their vertical distribution in the open lake over a 24-hour period. Between 10 AM and 4 PM, only a small proportion (5.5 ± 2%) of the gammarids were found in the upper .5 m of water, which, according to his Secchi-disc readings, was well illuminated. Most of the gammarids (77.6 ± 3.8%) were near the bottom, which his Secchi-disc readings suggest had little or no illumination. A significantly higher proportion of the gammarids (23.3 ± 3.1%) were found in the upper .5 m between 10 PM and 4 AM. My laboratory studies suggest that this distribution is largely determined by their strongly photophobic behaviour and the availability of benthic food sources. Similar factors affect the distribution of *H. acteca* (Hargrave 1970).

The distribution, light responses, and feeding habits of gammarids infected with *L.m.*, *L.* spp., and *P.p.*A were indistinguishable from those of the uninfected amphipods (Table 13). Although the light responses of *P.c.*-infected gammarids were not tested experimentally, no differences were detected during general observations on their distribution in the study lakes and laboratory aquaria.

However, the tests and observations revealed distinctly different behaviour in gammarids infected with *P.p.* and *P.m.*, and hyalellids infected with *C.c.* The tests also demonstrated that amphipods infected with each species show different and specific combinations of altered responses. This is illustrated in Table 13, which summarizes the comparative responses of the amphipod groups to the various tests.

A photophilic response was demonstrated in all three groups. In the *P.p.*-infected gammarids and *C.c.*-infected hyalellids, the response was sensitive to light intensity; these amphipods remained in the area with the

Table 13. Comparative responses of infected and uninfected amphipods during a series of light and disturbance tests

	Preferred zone			Phototaxis		Phototaxis after disturbance	Clinging
	DZ*	LZ*	ULZ*	Illumination gradient	Alternating light		
Gammarus lacustris							
Uninfected	+	-	R	-	-	-	-
Polymorphus paradoxus	-	+	+	+	+	+	+
P. paradoxus acanthellae	+	-	R	NT	-	-	-
P. marilis	-	+	R	+	-	-	-
Lateriporus mathevossianae	+	-	R	NT	-	-	-
Lateriporus spp. (includes *L. clerci* and *L. skrjabini*)	NT	NT	NT	NT	-	-	-
Hyalella azteca							
Uninfected	+	-	-	NT	-	-	-
Corynosoma constrictum	-	+	+	NT	+	+ or -**	-

R = Randomly distributed; NT = Not tested.

*DZ = Dark Zone; LZ = Light Zone; and ULZ = Upper 3 cm of Light Zone of a light-dark choice aquarium.

**See text for detailed explanation.

highest level of illumination for most of the testing. The photophilic behaviour of gammarids infected with *P.m.* was different in that it was apparently not sensitive to intensity; these gammarids showed no selection for the area of most intense illumination, but were highly selective for the illuminated area in general. The *P.m.*-infected gammarids obviously preferred the lighted zone, and often entered the area nearest to the source of illumination, but did not remain in that area.

The photophilic behaviour of *P.p.*-infected gammarids and *C.c.*-infected hyalellids may influence other behaviour patterns such as their foraging and feeding activities. Although no specific studies were made of their foraging and feeding behaviour, general observations suggested that they foraged throughout the entire aquarium, but particularly in the surface area. On several occasions during the study, *P.p.*-infected gammarids were observed to capture food items and then carry them to floatage or other surface material rather than to the bottom to feed. The resulting distribution of their foraging and feeding activities was quite different from that of the uninfected gammarids.

Based on his 24-hour distribution studies Mennon (1966) suggested that *G. lacustris* has a diel periodicity of activity, with the greatest activity occurring shortly before noon, and minimal activity in the late afternoon and dark hours. His observations on gammarids kept in the laboratory showed a similar rhythm. My general observations on the activity of uninfected and infected amphipods in the laboratory revealed the same rhythm. In addition, observations suggested that the number of *P.p.*-infected gammarids found clinging after disturbance was actually a measure of the numbers feeding or resting at the surface before disturbance.

The significantly larger proportion clinging to surface material in the afternoon (3-4 PM), when Mennon's observations showed a decrease in activity, can be interpreted as an increase in the numbers resting on floating material. The photophilic behaviour of the *P.p.*-infected gammarids suggests that they would select such resting places.

Nearly all of the *P.p.*-infected gammarids, and a majority of the *C.c.*-infected hyalellids, were positively phototactic in the absence of disturbance. The initial responses of the *P.m.*-infected gammarids in the horizontal gradient of illumination suggested that these were also positively phototactic. When subjected to alternating light from above and below, however, their phototaxes were strongly negative. On the assumption that the latter test subjects the phototactic behaviour of the amphipods to a more critical evaluation, the *P.m.*-infected gammarids were considered to be negatively phototactic.

It is difficult to test directly the effects of altered light responses on the micro-distribution of *P.p.*- and *P.m.*-infected gammarids and *C.c.*-infected hyalellids in the lakes, since sampling techniques create a disturbance and elicit other responses in the amphipods. However, the results of the laboratory studies indicate that, because of their altered photic behaviour, most of the *P.p.*-infected gammarids and *C.c.*-infected hyalellids would be distributed in the surface area, and that the *P.m.*-infected gammarids would be distributed throughout the lighted zones of the lakes.

Evasive Responses

The disturbance tests show that *G. lacustris* and *H. azteca* utilize light in directing the course of their evasive response. Under normal

(overhead) lighting conditions, the uninfected amphipods dive to the bottom, where they often burrow into the sediment or crawl under debris. When the light direction is reversed, the response is reversed, bringing the amphipods to the surface where they "dig" at the air-water interface with their gnathopods. This latter action is identical in appearance to the "skimming" of *P.p.*-infected gammarids, and appears to be a direct parallel to their usual response of burrowing into the sediment. The evasive behaviour of gammarids infected with *P.p.*A, *P.m.*, *P.c.*, *L.m.*, and *L.* spp. was identical to that of uninfected amphipods. The negative phototaxis of the *P.m.*-infected gammarids was equally intensified by disturbance indicating that their normally photophilic behaviour does not influence their evasive response.

The response of *C.c.*-infected hyalellids to disturbance was more complex. A significant proportion was consistently positively phototactic, both before and after disturbance. However, the number showing negatively phototactic responses was significantly increased after disturbance (Table 10); many which had previously responded positively to the light responded negatively after disturbance.

Disturbance had a dramatic effect on the behaviour of the *P.p.*-infected gammarids, eliciting an intense evasive response. Their usually positive phototaxis was intensified, and almost every individual showed a strong positive response. The positive phototaxis leads them to the surface, and brings on the skimming, obviously a modified burrowing response (cf the response of disturbed uninfected gammarids to light from below). The disturbance also triggers an additional response--clinging.

The precursor of this behaviour is unclear, but it may be

associated with an inability of the gammarid to complete its evasive response by burrowing into the substrate. The "clinging-like" behaviour of uninfected gammarids trapped in a net may be another manifestation of this reaction. The usual sequence (rising to the surface, skimming, then clinging) can be short-cut at any stage if the gammarid encounters something to cling to. In addition to the more intense response to disturbance, these gammarids tend to be more sensitive, responding to relatively minor disturbances.

The specificity of the altered response and the consequent micro-distributions of amphipods infected with *P.m.*, *C.c.*, and *P.p.* are obviously magnified by these differences in evasive behaviour.

Ontogeny of Altered Behaviour

It is apparent that young cystacanths (1-7 days old) of *P.p.* are not infective to the definitive host and that gammarids harbouring them do not show altered behaviour patterns. As soon as the altered behaviour appears, the cystacanths are infective. The small number of gammarids harbouring cystacanths of *P.p.*, but showing normal behaviour, may have been tested during this pre-maturation period.

The same explanation cannot be used to account for the varied responses of *C.c.*-infected hyalellids. Some of the infected hyalellids which had been kept in the laboratory for several months showed normal responses when tested, while others which were known to have recent infections showed altered responses. Nor was there any relation between intensity of infection and occurrence of the altered behaviour. Many of the positively phototactic individuals contained only single cystacanth infections.

Possible Mechanisms for Behavioural Changes

The possible mechanisms involved in the development of the behavioural changes were not specifically investigated in this study. Holmes and Bethel (in press) reviewed several systems in which parasite-induced altered responses to environmental stimuli were probable. In no case has the mechanism been investigated.

In the only other system in which altered responses to specific environmental stimuli have been demonstrated, i.e., *Dicrocoelium dendriticum* metacercariae in formicine ants (see Introduction), the larvae are located in the tissue of the subesophageal ganglion, or "brain," of their hosts. Anokhin (1966) suggested that environmental conditions "apparently activate the larvae to exert physiological, mechanical, and other stimuli upon the ganglia, which in turn leads to the specific behaviour pattern of the infected ant." Because of their location, the metacercariae could affect the ganglion through direct mechanical action or through chemical means. In a similar system (*Brachylecithum mosquensis* metacercariae in carpenter ants), Carney (1969) found that metacercariae near, but not in, the supraesophageal ganglion of their hosts could cause behavioural changes, suggesting that mechanical action was not involved. Indeed, Hohorst (1964) suggested that the metacercariae of *D. dendriticum* may interfere with the neural center which regulates one aspect of their hosts' behaviour by upsetting the neural physiology, perhaps through metabolic products.

Whittaker and Feeney (1971) have raised the possibility that an allomone (a chemical produced by one species to evoke, in another species, physiological or behavioural reactions which are favourable to

the first species--Brown, Eisner, and Whittaker 1970) is involved in another system (plerocercoids of *Ligula intestinalis* in the coelome of various fishes, reviewed by Holmes and Bethel, in press).

Applying these hypotheses to the amphipod-cystacanth systems, it is apparent that mechanical factors are not involved, since the cystacanths float freely in the haemocoele. The possibility of an allomone-mediated system is more likely.

Normally, cystacanths are completely enclosed by a thin connective tissue envelope of host origin (Crompton 1970, this study). When this envelope is absent or damaged the larvae are unable to survive, and are encapsulated by host haemocytes (Crompton 1967, Robinson and Strickland 1969). Of the hundreds of *P. paradoxus* cystacanths examined in this study, four were missing the envelope and one had a damaged envelope. All five were encapsulated by haemocytes, had deposits of melanin around them, and were presumably either dead or moribund. None of the gammarids harbouring these cystacanths showed any signs of altered behaviour, suggesting that a living, metabolizing parasite is necessary to produce the behavioural alterations.

Two attempts were made to test this hypothesis. The first involved surgical removal of the cystacanth. Because of its large size, it proved to be impossible to remove it without killing the host. The second involved an attempt to damage the envelope *in situ* (with a micro-needle) and evoke a haemocytic encapsulation. The envelopes proved to be remarkably resistant and all attempts to puncture them failed.

THE VULNERABILITY OF INFECTED AND UNINFECTED AMPHIPODS TO PREDATION

The behavioural alterations which are induced in the amphipod hosts by cystacanths of *P.p.*, *P.m.*, or *C.c.* have the overall effect of placing the amphipods in micro-distributions different from those of each other or from those of uninfected amphipods. The original intent of this portion of the study was to test each of these systems for vulnerability to predation by mallards, lesser scaup, and muskrats. Unfortunately, in the laboratory tanks, the laboratory-reared scaup appeared to be disturbed, did not display their normal feeding and diving behaviour (as described by Sugden, 1969, 1971), and made no attempt to feed on the amphipods. Tests involving scaup, therefore, were meaningless and were not continued. However, the mallards and muskrats readily adjusted to the experimental conditions, appeared to feed normally, and were remarkably easy to observe at relatively short distances. They were used in two series of experiments. Each experiment consisted of several tests in which a combination of infected and uninfected amphipods were exposed to predation by one or two predators for various time periods.

In the first experiment, six tests were conducted with *P.p.*-infected and uninfected gammarids (up to 75 each) and one or two mallards. Tests 1-4 were performed outside in the shallow tank; tests 5 and 6 were done in the laboratory observation room in the deeper tank. The gammarids were allowed at least 10-15 minutes to settle in the tanks before the mallards were allowed to enter the water. New, naive mallards were used in each test. Some were more at ease and performed better when used in pairs.

In each case, the mallards were first attracted to the floating material and fed around the floatage before dabbling or tipping. When the floating material was disturbed, most of the *P.p.*-infected gammarids remained clinging, although a few skimmed away along the surface. Mallards fed first on the clinging gammarids, then on any skimming in the immediate vicinity. The skimming appeared to be very attractive to mallards, and they rarely missed gammarids which were skimming along the surface. The mallards repeatedly returned to the floatage, turned it over and fed on any gammarids present. They also struck at any small objects (including bubbles) on the water surface. All of these activities were recorded on film.

The mallards were often allowed to re-enter the tanks after the completion of the test, while the surviving gammarids were being counted. During these periods, and at the ends of some of the tests, they repeatedly inspected the floatage and consumed much of the water plants and reeds which had been used. In each test, the mallards ate a significantly greater number of gammarids infected with *P.p.* (determined by Chi-square contingency tests); over the entire series, over four times as many infected gammarids were eaten (Table 14).

In a second experiment, 50 each of *P.p.*-infected, *P.m.*-infected, and uninfected gammarids were placed in the deeper tank, outside, along with one naive mallard. Two such tests, each lasting 10 minutes, were run. The behaviour of the mallards was identical to that in the first experiment; about 40% of the *P.p.*-infected gammarids, but not a single *P.m.*-infected or uninfected gammarid, was eaten (Table 15).

In a third experiment, 50 *C.c.*-infected and 50 uninfected

Table 14. Vulnerability of *Gammarus lacustris* infected with *Polymorphus paradoxus* to predation by mallard ducks

Test No.	Duration (min)	No. ducks	Gammarids eaten		P
			Uninfected	Infected	
1	7	2	6/25*	16/25	<.005
2	5	2	13/50	35/50	<.0005
3	5	2	12/50	42/50	<.0005
4	5	1	8/50	18/50	<.025
5	10	1	0/75	48/75	<.0005
6	15	1	24/75	63/75	<.0005
Total			63/325	222/325	<.0005

*Number of gammarids eaten/number available.

Table 15. Comparative vulnerability of uninfected *Gammarus lacustris* and those infected with *Polymorphus paradoxus* and *P. marilis* to predation by mallard ducks

Test No.	Duration (min)	No. ducks	Gammarids eaten		
			Uninfected	*P. paradoxus*	*P. marilis*
1	10	1	0/50	22/50	0/50
2	10	1	0/50	17/50	0/50
Total			0/100	39/100	0/100

hyalellids were placed in the deeper outdoor tank, along with one naive mallard. Two such tests were run, one lasting 15 minutes, the other 30 minutes. The behaviour of the mallards in these tests was considerably different from that of the mallards in the first two experiments. They promptly inspected the floating objects, but did not return to them except to feed on the *Ceratophyllum* sp. or *Myriophyllum* spp. They seldom dabbled or dipped and generally did not appear to be stimulated to search for food. The mallards engaged in other, casual, activities, such as bathing and playing, for a much greater proportion of these tests. In the previous tests, such activities comprised a small percentage of the testing time, and were usually displayed only prior to their feeding on *P.p.*-infected gammarids.

The only hyalellids eaten by mallards were ones infected with *C.c.*, and the numbers of these which were consumed--6 in the first and 10 in the second test--were relatively low. The hyalellids were very difficult to see in the tanks; as a result I never was able to observe the actual ingestion of the infected hyalellids. They may have been ingested when the mallards fed on the aquatic plant material.

An additional possibility, that the red spot produced by the presence of the cystacanth (such as in Fig. 2, p. 9) increases the conspicuousness of infected amphipods, and that the spot alone may play an important role in the predator-prey relationships of the amphipods with mallards, was tested in a fourth experiment. Red oval marks, 1 x 1½ mm, approximately the size and color of the largest cystacanth (*P.p.*), were painted on the dorsal-lateral aspect of the cuticles of a group of uninfected gammarids. The paint was fast drying and did not

affect the health, behaviour, or swimming ability of the gammarids, as determined by preliminary observations. Fifty uninfected, marked gammarids and 50 without the marks were placed in the deeper tank, along with one mallard. Four 10-minute tests were run, the first two tests outside. The mallards' behaviour was identical to that of the mallards in the tests with *C.c.*-infected hyalellids. They did not appear to feed actively on the gammarids; very few were eaten, and the proportions of marked and unmarked gammarids that were eaten were not significantly different (Table 16).

The second series of experiments was designed to determine the susceptibility of infected and uninfected gammarids to ingestion by muskrats, and to determine the method(s) by which the gammarids are ingested (i.e., by active predation, or by accidental ingestion, or both). In three tests, 75 *P.p.*-infected and 75 uninfected gammarids were exposed to one muskrat for 24 hours. All of the tests were performed outside in the deeper tank, which had been modified to produce as natural conditions as possible for the muskrats.

The muskrats were most active in the tank in the late afternoon, evening, and early morning. During the late-afternoon periods, they gathered most of the floating vegetation and returned to the box to feed. The animals usually entered the box through the front or bottom entrance and immediately began feeding on the material. It was usually possible to observe the feeding through a side entrance of the box. On three occasions (in the second and third tests), I observed the muskrats ingesting *P.p.*-infected gammarids while feeding on *Ceratophyllum* sp. and *Myriophyllum* spp. The gammarids were obviously clinging to the leaves

Table 16. Vulnerability of marked and unmarked *Gammarus lacustris* to predation by mallard ducks

Test No.	Duration (min)	No. ducks	Gammarids eaten	
			Marked	Unmarked
1	10	1	3/50	2/50
2	10	2	5/50	1/50
3	10	1	0/50	0/50
4	10	1	0/50	0/50
Total			8/200	3/200

of the plants; the cystacanths of *P.p.* were easily recognizable in most of these gammarids. In all three tests, some *P.p.*-infected gammarids were found among plant material which the muskrats had moved to the upper level of the box. Although no uninfected gammarids were eaten, significant numbers of *P.p.*-infected gammarids were eaten in each of the three tests (Table 17). From the observations given above, it is logical to assume that all of these were accidentally ingested with plant material. The muskrats never appeared to be attracted by swimming, skimming, or clinging gammarids, and were never observed to pursue or intentionally feed on a gammarid. Several *P.p.*-infected gammarids were usually clinging onto the wooden stick in the water, which was completely ignored by the muskrats.

In each of the two subsequent tests, 50 *P.m.*-infected and 50 uninfected gammarids (a sufficient number of *P.p.*-infected gammarids were not available) were placed in the modified deep tank with one muskrat for 24 hours. The behaviour of the muskrats was identical to that of the muskrats in the preceding experiment, but none of the gammarids, either uninfected or those infected with *P.m.*, were eaten. All were recovered alive at the end of each test.

Table 17. Vulnerability of *Gammarus lacustris* to accidental ingestion by muskrats

Test No.	Duration (hr)	No. muskrats	Gammarids eaten	
			Uninfected	Infected
1	24	1	0/75	16/75
2	24	1	0/75	25/75
3	24	1	0/75	17/75
Total			0/225	58/225

DISCUSSION

Amphipods are never eaten extensively by mallards (Chura 1961), even when the amphipods are abundant in the environment (Perret 1962). The predator tests indicate that this is due to a combination of normal behaviour patterns of the amphipods and feeding behaviour of the mallards. Mallards are dabbling ducks, and feed chiefly by dabbling on the water surface, picking out food items by visual means (Perret 1962, my observations) and apparently feeding underwater only when surface items are not available. However, tipping is an important auxiliary feeding method, especially in shallow areas where they are able to reach the bottom. The initial reaction of all 18 mallards was to inspect the floating material before dabbling or tipping. Since all were laboratory raised, fed completely on a mash diet, and were never previously exposed to natural aquatic invertebrate foods, this method may be an innate feeding mechanism additional to those described by Weidmann (1956). This method may be an important component of the trial and error method for learning which foods are edible as suggested by Perret (1962). The normal photophobic behaviour of the amphipods removes them from this feeding niche of the mallards. Their evasive response to the disturbance created by a feeding mallard would make them even less accessible.

On the other hand, the predator tests show that gammarids infected with *P.p.* are very vulnerable to predation by mallards. The difference appears to be that the abnormal photophilic behaviour of the infected gammarids places them in a new micro-habitat, one within the feeding niche of the ducks. In this micro-habitat, the gammarids are also more

susceptible to being disturbed. Because of their greater sensitivity, even the slightest disturbance would elicit the skimming and attract the attention of any mallard feeding nearby. The clinging behaviour may have two complementary consequences. It is reasonable to assume that mallards accidentally ingest some *P.p.*-infected gammarids with aquatic vegetation, on which they feed extensively. Gammarids clinging among the dense foliage of *Ceratophyllum* sp. and *Myriophyllum* spp. are well hidden, and could easily be ingested without being seen. In addition, following predator tests, infected gammarids were seen clinging onto the feathers of ducks on three occasions, so that it is also possible that mallards ingest some *P.p.*-infected gammarids while preening.

A more important consequence of the clinging behaviour may be the establishment of a search image in the ducks. The ducks repeatedly returned to inspect the floatage, but only after being initially successful in sighting and consuming *P.p.*-infected gammarids clinging to the material. They never displayed this behaviour during tests in which *P.p.*-infected gammarids were not present. The response had the characteristics Croze (1970) described for Tinbergen's (1960) "specific searching image": it involved a change in the searching pattern (the repeated returns to the floating material), it was quickly acquired from relatively few encounters with clinging amphipods, it took advantage of high densities of prey associated with a particular background (the floating material), and it persisted through periods of no reward (at the end of the test), but was lost after longer periods (overnight) when the birds were fed a different food.

The ducks also repeatedly struck at any small objects on the water.

This reaction may have been due to the formation of a second search image for skimming gammarids. The association in this case might be with the disturbance of the water created by the skimming. All of the search images enhance the food-searching activity of the ducks, which, in turn, creates more disturbance, more reactions by infected gammarids, and thus a positive feedback loop which maintains a strong searching stimulus in the ducks.

The cystacanths of *P.p.* are bright to dark red, and very conspicuous, making their amphipod hosts stand out clearly (my observations and those of others working at the study lakes). In fact, Barrett and Butterworth (1968) suggested that the bright orange cystacanths of *Polymorphus minutus* in the haemocoeles of their hosts, *G. pulex*, may facilitate their transfer to the definitive hosts (mallards). Although the predator tests showed that the vulnerability of uninfected gammarids is not affected by the presence of the red spots, it is difficult to evaluate the significance of the color in the absence of the behavioural alterations. For example, the contrast created by the red outline of *P. paradoxus* cystacanths in the darker background of the gammarid hosts (Fig. 2) may increase the conspicuousness of clinging gammarids, particularly when the color of the amphipods blends well with that of the object to which they are clinging. Kear (1964) has demonstrated that many ducks, both dabblers and divers, show a preference for pecking at green objects. But, as Croze (1970) pointed out, searching images must direct "omnivorous animals in particular . . . [to] react in spite of their innate preferences, or whole classes of potential prey will remain unexploited."

The experiments with the muskrats indicate that the clinging

behaviour of *P.p.*-infected gammarids is totally responsible for their increased vulnerability to ingestion by muskrats. Furthermore, the ingestion of the infected gammarid in this system appears to be completely accidental. In view of their normal feeding habits (Errington 1963, Bradt 1938), the likelihood of either muskrats or beavers intentionally feeding on an arthropod the size of *G. lacustris* is highly improbable. Two of the muskrats which were used were trapped at Hastings Lake, and had undoubtedly been exposed to large numbers of gammarids, yet their behaviour during the tests was identical to that of a muskrat from Wabamun Lake, which has a comparatively small population of the amphipods.

Thus, *P. paradoxus* has adopted the strategy, illustrated in Fig. 1, of altering the responses of its intermediate hosts to environmental stimuli (in this case, light) in such a way as to move the animals into the zone of overlap with the feeding niche (in this case, the upper lighted zone of the lake) of the definitive hosts. In the present study, I have demonstrated the overlap, and that infected gammarids are significantly more vulnerable to predation and/or accidental ingestion by two of the definitive hosts of the parasite. The efficiency of the strategy, that is, the extent to which the behavioural alterations make the infected amphipods vulnerable only to suitable definitive hosts, was not tested in this study due to the difficulties encountered in using laboratory-reared scaup.

The strategies adopted by *P. marilis* and *C. constrictum* are less obvious, perhaps because neither system was studied in the same detail as that of *P. paradoxus*. Lesser scaup, the main host for *P. marilis*, feed to a considerable extent on amphipods (Dirschl 1969, and references therein); with the greater degree of overlap between the habitat of the

amphipods and the feeding niche of scaup, less drastic changes in the behaviour of infected gammarids would be sufficient for *P. marilis*. Since scaup feed chiefly by diving in open water (Sugden 1969), no attraction to the surface would be required, and a simple change to a photophilic pattern might be an adequate strategy.

Corynosoma constrictum has a wider host range than the other two species (Table 1), and the variable behaviour patterns exhibited by infected hyalellids, particularly after disturbance, may reflect that fact. Their photophilic and phototactic responses, along with a predilection for vegetation, would bring them to the surface of the vegetated areas, where they would be available to surface feeders, such as mallards, or to scaup, which hesitate to dive through vegetation (Sugden 1965), but will feed at, or just under, the surface of heavily vegetated areas (my observations). Those which dive after disturbance may be more vulnerable to bottom-feeders, such as pintails (*Anas acuta*) (Sugden 1965). It would be very interesting to test their vulnerability to ducks using these other feeding methods.

Selective predation on infected individuals is of obvious advantage to the parasite, but is it not also advantageous to the prey population? Many of the best examples of effects on the behaviour of infected intermediate hosts (reviewed by Holmes and Bethel, in press) involve parasites that do considerable damage to the host, or sterilize it, or both. The destruction of host gonads by plerocercoids of *Ligula intestinalis* and *Schistocephalus solidus* is well known (reviewed by Williams, 1967, Arme and Owen 1967, 1968); *Leucochloridium*, like many other trematodes, effectively castrates its host (Wesenberg-Lund 1931);

my observations, and those of others in the parasitology group, indicate that cystacanths of *Polymorphus paradoxus* also castrate their hosts. Behavioural changes produced by dicrocoelid metacercariae in ants would eliminate those individuals as productive members of the ant society; infected ants are obviously poorer foragers than uninfected ants (see Introduction, pp. 4 and 5). Therefore, predation on infected individuals is predation the excess, expendable individuals.

In addition, there may be another advantage accruing to the prey population. If there are sufficient infected individuals, predators might be expected to develop search images dependent upon the behavioural peculiarities of those infected individuals, as in the mallards in this study and possibly the robins in Carney's (1969) study. Such search images would be protective of the reproductive and productive individuals of the prey populations.

The "knowledge" and "diabolical cunning" which Professor LaRue (see Introduction, p. 1) related to the methods utilized by parasitic helminths for enhancing transmission into the various hosts of their life cycles, is perhaps best exemplified by the evolutionary strategies described by Holmes and Bethel (in press). The development of these strategies is, of course, the result of long evolutionary adaptation through many generations of successful life cycles. Such adaptations by parasites are to be expected; the extent to which they are used, however, is difficult to assess due to the lack of thorough investigations.

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A P P E N D I X

Appendix 1. Time spent by infected and uninfected amphipods in different areas of a light-dark choice aquarium

	Time, in seconds, spent in:			
	ULZ*	RLZ*	TLZ*	DZ*
Gammarus lacustris				
Uninfected	125	210	335	3265
	0	0	0	3600
	0	0	0	3600
	0	0	0	3600
	0	0	0	3600
	0	0	0	3600
	0	0	0	3600
	0	0	0	3600
	34	120	154	3446
	0	76	76	3524
Total	159	406	565	35435
Polymorphus paradoxus cystacanths	3520	80	3600	0
	3152	310	3462	138
	2973	432	3405	195
	3600	0	3600	0
	3196	120	3316	284
	2780	120	2900	700
	1232	0	1232	2368
	1305	2115	3420	180
	1935	1027	2962	638
	2460	1140	3600	0
Total	26153	5344	31497	4503
P. paradoxus acanthellae	0	0	0	3600
	128	505	633	2967
	0	0	0	3600
	0	0	0	3600
	125	60	185	3415
	0	0	0	3600
Total	253	565	818	20782

Appendix 1 (continued)

	Time, in seconds, spent in:			
	ULZ*	RLZ*	TLZ*	DZ*
P. marilis	180	3420	3600	0
	209	3391	3600	0
	370	3230	3600	0
	83	3517	3600	0
	234	3366	3600	0
	615	2985	3600	0
Total	1691	19909	21600	0
Lateriporus mathevossianae	0	0	0	3600
	35	170	205	3395
	350	445	795	2805
	126	275	401	3199
	60	230	290	3310
Total	571	1120	1691	16309
Hyalella azteca				
Uninfected	0	0	0	3600
	0	0	0	3600
	0	0	0	3600
	0	0	0	3600
	0	0	0	3600
	0	0	0	3600
	0	0	0	3600
	0	0	0	3600
	0	0	0	3600
	0	0	0	3600
	0	0	0	3600
	0	0	0	3600
Total	0	0	0	43200

Appendix 1 (continued)

	Time, in seconds, spent in:			
	ULZ*	RLZ*	TLZ*	DZ*
Corynosoma constrictum	3600	0	3600	0
	3300	300	3600	0
	3537	63	3600	0
	3600	0	3600	0
	3600	0	3600	0
	3600	0	3600	0
	3600	0	3600	0
	3600	0	3600	0
	3600	0	3600	0
	3600	0	3600	0
	3600	0	3600	0
	3600	0	3600	0
Total	42837	363	43200	0

*ULZ = Upper 3 cm of Light Zone; RLZ = Remainder of Light Zone; TLZ = Total Light Zone; DZ = Dark Zone.